UDC: 638.138(497.75)"2023/2034"
Professional paper

NUTRITIONAL VALUE OF HONEY BEES-COLLECTED POLLEN IN DIFFERENT SEASONS OF THE YEAR

Hrisula KIPRIJANOVSKA[1], Nusmir RAMADANI[1], Srekjko GJORGJIEVSKI[1]
[1] *Faculty of Agricultural Sciences and Food, Skopje, Ss. Cyril and Methodius University in Skopje, Republic of North Macedonia*
[*] *Corresponding Author: e-mail:* hrisulak@yahoo.com

Abstact

This study analyzes the nutritional value of pollen collected by honey bees, focusing on crude protein and fat content in relation to bee dietary requirements. Pollen samples were collected from two apiaries situated in distinct environments (lowland and semi-mountainous) within the Polog region of the Republic of North Macedonia. Bottom pollen traps were placed on five experimental colonies at each site, and collections occurred from July 1, 2023, to June 30, 2024. Sampling was conducted biweekly, with traps activated for up to 72 hours. During each sampling, pollen from all five colonies per site was pooled, labeled, and stored at −18°C until laboratory analysis. Chemical composition was analyzed using monthly pooled samples, which were grouped by season (spring, summer, autumn). Crude protein was determined using the Kjeldahl method (MKC EN ISO 5983-1:2010; MKC EN ISO 5983-1:2010/AC:2010), and fat content was measured according to MKC ISO 6492:2012. Protein content varied significantly by season, peaking in spring (22.29 ± 2.12%) and summer (21.38 ± 2.73%), and declining markedly in autumn (16.61 ± 2.46%). ANOVA confirmed a significant seasonal effect on protein content, with post hoc tests indicating that autumn levels were significantly lower than both spring and summer. In contrast, fat content showed no significant seasonal differences.

Keywords: pollen trap, honey bee, crude protein, fat, seasonal variation

1. Introduction

Honey bees (*Apis mellifera*) play a critical role as pollinators in both natural ecosystems and agricultural landscapes, contributing to biodiversity and crop productivity (Klein et al., 2007). The health and productivity of bee colonies depend heavily on access to nutritionally adequate forage—especially pollen, which serves as the primary source of protein and lipids (Brodschneider & Crailsheim, 2010). Proteins are essential for larval development, enzyme production, and immune function, while lipids support energy storage, cell membrane integrity, and hormonal regulation (Arien et al., 2015; Di Pasquale et al., 2013).

The nutritional composition of pollen varies widely among plant species and is influenced by environmental conditions, geography, and seasonality (Roulston & Cane, 2000). As flowering phenology changes throughout the year, both the botanical diversity and nutritional quality of available pollen shift, leading to seasonal variations in protein and fat content (Di Pasquale et al., 2013; Tasei & Aupinel, 2008). These changes may create periods of nutritional surplus or deficit, affecting brood development, overwintering success, and colony resilience (Alaux et al., 2010).

Although the importance of pollen quality is increasingly recognized, relatively few studies have quantified seasonal dynamics in macronutrient content—particularly within specific regions or climates. Such information is essential for beekeepers to make informed decisions about supplemental feeding and for landscape management strategies to ensure adequate floral resources throughout the foraging season (Donkersley et al., 2014; Vaudo et al., 2015).

This study investigates seasonal variation in the protein and fat content of bee-collected pollen in the Polog region, aiming to identify temporal nutritional patterns and potential seasonal gaps.

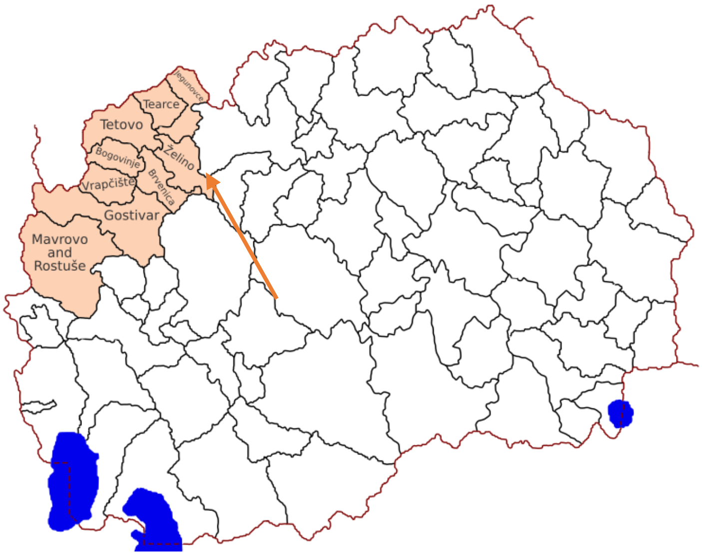
https://doi.org/10.62792/ut.jasrd.v3.i5-6.p3014

By providing insight into macronutrient fluctuations over time, this research contributes to a deeper understanding of honey bee nutrition.

2. Materials and methods

Study Area and Sampling Periods

The study was conducted in the Polog region of the Republic of North Macedonia, comprising nine municipalities (see Map 1). Pollen was collected from two apiaries in the municipality of Zhelino—one in the village of Zhelino (400 m altitude) and the other in the village of Chiflik (70 m altitude). Sampling took place from July 1, 2023, to June 30, 2024, excluding the winter months.



Map 1 Study area (Polog region in of North Macedonia, municipality of Zhelino)

Pollen Collection

Pollen traps were installed at the entrances of five experimental hives at each site. Sampling occurred every 14 days, with traps activated for up to 72 hours. A total of 28 samples were collected. Due to unfavorable weather in April and May, only one collection was made per month. In November, no pollen was collected due to colony inactivity (the final autumn sample was collected on October 31, 2024). At each sampling, pollen from all five colonies at a site was pooled, labeled, and stored frozen until analysis.

Nutritional Analysis

Pollen samples were dried and homogenized before analysis. Nutritional testing was conducted on pooled monthly samples, which were then grouped by season (spring, summer, autumn). Crude protein content was determined using the Kjeldahl method (MKC EN ISO 5983-1:2010; MKC EN ISO 5983-1:2010/AC:2010), and fat content was measured following the MKC ISO 6492:2012 protocol.

Data Analysis

Seasonal differences in protein and fat content were analyzed using one-way ANOVA followed by Tukey's post hoc tests. Statistical significance was set at $p < 0.05$.

Results

The results of the protein content in pollen are presented in Table 1, Figure 1 and Table 2.

Table 1. Protein content (%) in bee-collected pollen across seasons

Season	Mean ± SD (Protein %)	Min–Max
Spring	22.29 ± 2.12	18.59–25.61
Summer	21.38 ± 2.73	16.97–24.34
Autumn	16.61 ± 2.46	13.06–20.61

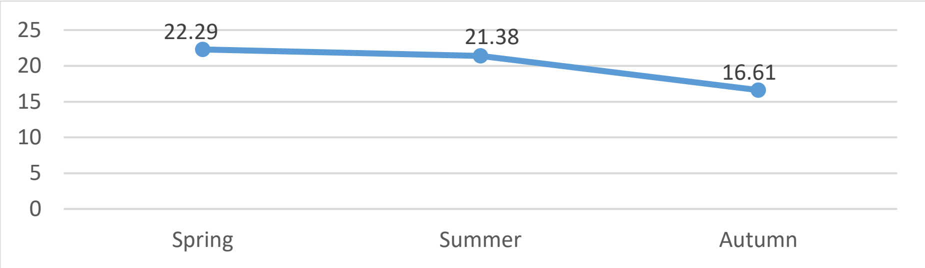


Figure 1 Protein Content (%) in bee-collected pollen across seasons

Table 2. One-way ANOVA results for protein content (%) in bee-collected pollen

Source of Variation	Sum of Squares	d.f.	Variance	F	p-value
Between Groups	154.5918	2	77.2959	12.4028	0.0002
Within Groups	155.8039	25	6.2322		
Total	310.3957	27			
Post-hoc tests					
Tukey HSD Post-hoc Test... Group 1 vs Group 2: Diff=-0.9100, 95%CI=-3.7482 to 1.9282, p=0.7073 Group 1 vs Group 3: Diff=-5.6800, 95%CI=-8.7891 to -2.5709, p=0.0003 Group 2 vs Group 3: Diff=-4.7700, 95%CI=-7.6082 to -1.9318, p=0.0009					

The results of the fat content in pollen are presented in Table 3, Figure 2 and Table 4.

Table 3. Fat content (%) in bee-collected pollen across seasons

Season	Mean ± SD (Fat %)	Min–Max
Spring	2.80 ± 1.37	1.25-5.08
Summer	2.25 ± 1.20	1.10-4.66
Autumn	2.36 ± 1.51	0.89-5.05

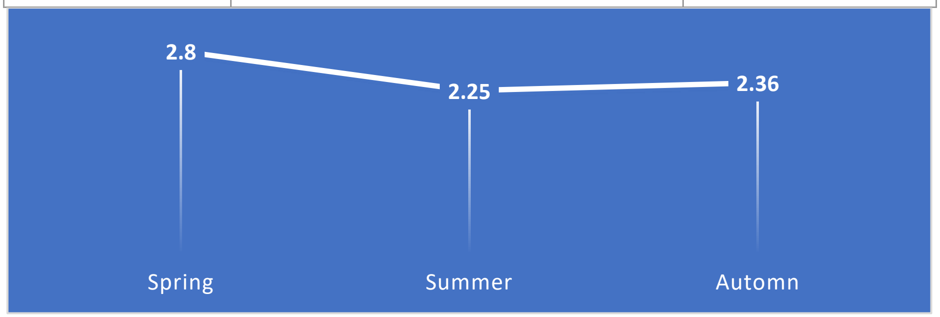


Figure 2 Fat content (%) in bee-collected pollen across seasons

Table 4. One-way ANOVA results for fat content (%) in bee-collected pollen

Source of Variation	Sum of Squares	d.f.	Variance	F	p-value
Between Groups	1.5211	2	0.7606	0.4231	0.6596
Within Groups	44.9390	25	1.7976		
Total	46.4601	27			
Post-hoc tests					
Tukey HSD Post-hoc Test... Group 1 vs Group 2: Diff=-0.5500, 95%CI=-2.0743 to 0.9743, p=0.6461 Group 1 vs Group 3: Diff=-0.4400, 95%CI=-2.1098 to 1.2298, p=0.7905 Group 2 vs Group 3: Diff=0.1100, 95%CI=-1.4143 to 1.6343, p=0.9824					

3. Discussion

The results revealed significant seasonal variation in pollen protein content, with the highest levels in spring (22.29 ± 2.12%), slightly lower in summer (21.38 ± 2.73%), and a substantial decline in autumn (16.61 ± 2.46%). These findings align with studies reporting that spring pollen tends to be richer in protein due to the abundance and diversity of floral resources (Di Pasquale et al., 2013). Somerville & Nicol (2006) and Tasei & Aupinel (2008) similarly found higher protein levels in spring pollen compared to later seasons.

Radev (2019) demonstrated seasonal protein variation across multiple years, with protein content ranging from 13.9% to 27.8% and an average around 21%, consistent with our results. Diana et al. (2014) found that pollen protein content exceeded 20% at two of three studied sites, meeting bee nutritional needs. Roulston & Cane (2000) noted that early-blooming species typically produce higher-protein pollen, supporting our observed autumn decline. Herbert & Shimanuki (1978) attributed late-season reductions to limited floral diversity and dominance of lower-protein species.

According to Shaw (1999), as cited by Saavedra-Carhuatocto et al. (2014), a minimum of 20% protein is necessary to meet honey bee nutritional requirements. Our findings show that spring and summer pollen met this threshold, while autumn pollen fell below it, indicating a potential risk for nutritional stress.

Based on Somerville’s (2001) classification of pollen quality:

- Poor (<20%)
- Average (20–25%)
- Above average (25–30%)
- Excellent (>30%)

Spring and summer samples were mostly in the average group, with some spring samples reaching above-average levels. Autumn samples were classified as poor quality. The presence of high-protein spring samples highlights the need to identify and preserve valuable floral sources.

In contrast to protein, fat content showed no significant seasonal variation ($F = 0.7606$, $p = 0.6596$). This supports findings by Human et al. (2007) and Somerville & Nicol (2006), who reported relatively stable fat levels across seasons. Somerville & Nicol’s study of 172 pollen samples yielded a mean fat content of 2.52%, closely matching our average (2.47%).

Di Pasquale et al. (2013) noted that while protein content varies with floral origin and season, fat is more closely tied to plant species. The stable fat content may reflect evolutionary constraints due to its role in pollen viability and germination. However, Singh et al. (1999) suggested that higher-fat pollen may be more attractive to bees. Lipids play essential roles in energy storage, royal jelly production, and nutrient metabolism.

4. Conclusions

Our findings confirm significant seasonal variation in the protein content of bee-collected pollen, with optimal levels in spring and summer and suboptimal levels in autumn. These fluctuations may impact colony health, particularly in autumn when protein needs may exceed supply. In contrast, fat content remained consistent across seasons.

These results emphasize the importance of ensuring continuous access to diverse and nutrient-rich floral resources. Supplemental feeding and habitat enrichment may be necessary during periods of protein scarcity to support colony development and survival.

References

[1] Alaux, C., Ducloz, F., Crauser, D. & Le Conte, Y. (2010) 'Diet effects on honeybee immunocompetence', *Biology Letters,* 6(4), pp. 562–565. https://doi.org/10.1098/rsbl.2009.0986

[2] Arien, Y., Dag, A., Zarchin, S., Masci, T. & Shafir, S. (2015) 'Omega-3 deficiency impairs honey bee learning', *Proceedings of the National Academy of Sciences,* 112(51), pp. 15761–15766.

[3] Arrese, E.L. & Soulages, J.L. (2010) 'Insect fat body: Energy, metabolism, and regulation', *Annual Review of Entomology,* 55, pp. 207–225. https://doi.org/10.1146/annurev-ento-112408-085356

[4] Brodschneider, R. & Crailsheim, K. (2010) 'Nutrition and health in honey bees', *Apidologie,* 41(3), pp. 278–294. https://doi.org/10.1051/apido/2010012

[5] Di Pasquale, G., Salignon, M., Le Conte, Y., Belzunces, L.P., Decourtye, A., Kretzschmar, A. *et al.* (2013) 'Influence of pollen nutrition on honey bee health: Do pollen quality and diversity matter?', *PLoS ONE,* 8(8), e72016. https://doi.org/10.1371/journal.pone.0072016

[6] Diana, T., Nicolson, S.W. & Human, H. (2014) 'Nutritional content of honey bee-collected pollen from *Eucalyptus grandis* and *Eucalyptus cladocalyx*', *Journal of Insect Physiology,* 70, pp. 1–7. https://doi.org/10.1016/j.jinsphys.2014.08.004

[7] Donkersley, P., Rhodes, G., Pickup, R.W., Jones, K.C. & Wilson, K. (2014) 'Honeybee nutrition is linked to landscape composition', *Ecology and Evolution,* 4(21), pp. 4195–4206. https://doi.org/10.1002/ece3.1293

[8] Herbert, E.W., Bickley, W.G. & Shimanuki, H. (1980) 'The brood-rearing capability of caged honey bees fed dandelion and mixed pollen diets', *Apidologie,* 11(1), pp. 23–29. https://doi.org/10.1051/apido:19800102

[9] Human, H., Nicolson, S.W., Strauss, K., Pirk, C.W.W. & Dietemann, V. (2007) 'Influence of pollen quality on ovarian development in honeybee workers (*Apis mellifera scutellata*)', *Journal of Insect Physiology,* 53(7), pp. 649–655.

[10] Klein, A.M., Vaissière, B.E., Cane, J.H., Steffan-Dewenter, I., Cunningham, S.A., Kremen, C. & Tscharntke, T. (2007) 'Importance of pollinators in changing landscapes for world crops', *Proceedings of the Royal Society B: Biological Sciences,* 274, pp. 303–313. https://doi.org/10.1098/rspb.2006.3721

[11] Radev, Z. (2019) 'The impact of different protein content of pollen on honey bee (*Apis mellifera* L.) reproduction', *New Knowledge Journal of Science,* 8(2), pp. 59–66.

[12] Roulston, T.H. & Cane, J.H. (2000) 'Pollen nutritional content and digestibility for animals', *Plant Systematics and Evolution,* 222(1–4), pp. 187–209. https://doi.org/10.1007/BF00984102

[13] Schmidt, J.O., Thoenes, S.C. & Levin, M.D. (1995) 'Survival of honey bees, *Apis mellifera* (Hymenoptera: Apidae), fed various pollen sources', *Annals of the Entomological Society of America,* 88(5), pp. 447–452. https://doi.org/10.1093/aesa/88.5.447

[14] Singh, R.P., Chandel, R.S. & Bhardwaj, N.R. (1999) 'Fat including fatty acids and sterols are important sources of energy used for the synthesis of reserve fat and glycogen and contribute to the production of royal jelly', *Journal of Apicultural Research,* 38(1–2), pp. 1–6.

[15] Somerville, D. & Nicol, H. (2006) *Crude protein and amino acid composition of honey bee-collected pollen pellets from south-east Australia.* RIRDC Publication No. 06/047.

[16] Somerville, D.C. (2001) *Nutritional value of bee collected pollens.* Rural Industries Research and Development Corporation, Publication No. 01/047.

[17] Tasei, J.N. & Aupinel, P. (2008) 'Nutritive value of 15 single pollens and pollen mixes tested on larvae produced by bumblebee workers (*Bombus terrestris,* Hymenoptera: Apidae)', *Apidologie,* 39(4), pp. 397–409. https://doi.org/10.1051/apido:2008017

[18] Vaudo, A.D., Tooker, J.F., Grozinger, C.M. & Patch, H.M. (2015) 'Bee nutrition and floral resource restoration', *Current Opinion in Insect Science,* 10, pp. 133–141. https://doi.org/10.1016/j.cois.2015.05.008