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Assessing the Impact of Chickpea (*Cicer Arietinum* L.), Prosopis Pods (*Prosopis Juliflora*), and Soybean (*Glycine Max*)-Based Diets on Bee Performance and Nutritional Value

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Abstract

The study evaluated the proximate composition of soybean (*Glycine max*), chickpea (*Cicer arietinum* L.), and Prosopis (*Prosopis juliflora*) pod meal-based bee diets. The three major ingredients were selected deliberately due to their availability and cost. Proximate analyses were conducted at Egerton University, Animal Nutrition laboratory. There were three experimental diets with three replicates each: T1 (soybean meal), T2 (chickpea), and T3 (ground Prosopis pods). Prosopis pods were harvested by plucking mature pods from the tree branches at the Kenya Agriculture and Livestock Research Organization (KALRO), ABIRI Centre (0°28'10.1"N, 35°58'59.79"E). They were dried and ground to pass through a 1mm screen. Proximate analyses were carried out following the AOAC guidelines. All variables were subjected to analysis of variance (ANOVA) in a completely randomized design (CRD) using the SAS 9.4 statistical package. The results indicated that there were significant differences at ($p < 0.05$) between the CP content of diets. Treatment (T1) (263.4 g/kg DM) and T2 (261.7 g/kg DM) had the highest CP, while T3 (250.6 g/kg DM) had the lowest compared to T1 and T2. Similar trends were observed in ash content, with T3 having the highest (39.1 g/kg DM), while T1 and T2 were not significantly different, with 36.5 g/kg DM and 34.4 g/kg DM, respectively. There were no significant differences ($p > 0.05$) in CF in all the treatments. There were significant differences in the fat content, with T1 having the highest, 73.4 g/kg DM, followed by T3 with 40.0 g/kg DM, while T2 had the lowest, with 31.2 g/kg DM. In the supplemental diets, T1, composed of 30% soybean, 45% sorghum flour, 10% skimmed milk, and 15% brewer's yeast, contained a significantly higher CP content compared to T2 and T3. This was in agreement with other studies that indicated that the inclusion of specific ingredients in a diet impacted CP levels in animal feed. From the chemical analyses, brewer's yeast had the highest crude protein content. Treatment T1, consisting of 30% soybean, 45% sorghum flour, 10% skimmed milk, and 15% brewer's yeast, had the highest fat content among the supplemental diets. The results of this study showed that a combination of soybean, sorghum, skimmed milk, and brewer's yeast mixed with honey was the best for bee feeding.

Keywords: Ash; Crude Protein; Dry matter; Honeybee; *Prosopis juliflora* pods

Introduction

Beekeeping contributes significantly to domestic income generation, environmental conservation via pollination services, apitherapy, and food and nutrition security on a global scale [1]. The consistent growth of the apiculture enterprise over time can be attributed to the implementation of contemporary beekeeping technologies [2]. The beekeeping industry has encountered several obstacles, including the depletion of nectar and pollen resources, the destruction of their natural habitat by human activities, and the application of hazardous chemicals in crop cultivation.

Globally, honeybees face several threats, many of which also affect local populations in Kenya. Some of the key challenges include; deforestation, urbanization, and agricultural expansion have led to a decline in bee habitats, reducing the availability of natural flora [3]. This habitat fragmentation limits bees' access to a diverse range of pollen and nectar sources, crucial for their health and reproduction. According to Silverman, (2024) the widespread use of pesticides, particularly neonicotinoids, has been linked to colony collapse disorder (CCD), where bee colonies die off suddenly. Pesticides affect bees' navigation, reproduction, and immune systems, making them more susceptible to diseases and parasites. Rising global temperatures and changing rainfall patterns affect flowering times and the availability of forage plants. Climate change also disrupts the delicate balance between bees and their ecosystems, making it harder for bees to find food and survive [4]. Across the globe, honeybees face a growing threat from diseases such as American foulbrood and parasites like the Varroa destructor mite [5]. These pathogens weaken colonies, leading to poor hive health and reduced honey production. In Kenya, the situation is worsened by additional region-specific challenges for example in Arid and semi-arid regions, which cover over 80% of Kenya's land area, are particularly vulnerable to prolonged droughts [6]. Water is a critical resource for bees, and during dry seasons, the availability of flowering plants drastically reduces, leading to food scarcity. This makes it difficult for bees to produce honey and maintain colony health.

The availability of high-quality pollen and nectar, which are essential for bee survival and development, is severely limited during dearth periods. Another component that impacts bee populations and well-being is bee feed [7]. A deficiency in bee forage may result in a decline in bee populations or the manifestation of indicators of nutritional stress. According to Abrol and Abrol [8], the honey bee is a good example of how nutritional stress can result in a variety of issues. Nutritional stress shortens the life span of honey bees and makes them less effective foragers [7]. Honey bees fed on a high-quality diet experienced less stress when exposed to *Nosema apis*, *Nosema ceranae*, and Varroa destructor. Assessing the potential use of locally sourced feed resources in honeybee nutrition is, hence, of the utmost importance [7].

Supplements for bees often contain soybeans. Bee dietary supplements usually include a combination of actual pollen, soy flour or protein isolate, yeast (to increase protein and vitamin B complex), vitamin and mineral supplements, sugar, honey, or oil (for additional fat), and sometimes other ingredients [9]. A larger concentration of pollen indicates a higher quality supplement. Grinding roasted soybeans into a fine powder produces soy flour, a low-carbohydrate, high-protein food product [5[9]. Different processing methods result in soy flour with different proximate compositions. When there is a lack of pollen, chickpea flour may be the best alternative to consider. Ghramh and Khan [10]found that chickpea flour is high in protein, carbohydrates, lipids, and moisture. It is necessary to evaluate the appropriateness of various flours with high protein and other critical constituents for bee diets, such as ground *Prosopis juliflora* pods.

The majority of bee research has relied on brewer's yeast in their formulations due to its high protein content, which is crucial for the bee colonies' activity, particularly during the dearth period [11]. The hypopharyngeal gland also relies on it during its development. A worker bee's hypopharyngeal gland develops in correlation with the amount of protein consumed and the bee's age [10]. Sorghum flour, when utilized as a supplemental feed, can be extremely beneficial to bee diets. According to Ghramh and Khan (2023), sorghum flour is an excellent source of carbohydrates, proteins, and a number of necessary minerals such as magnesium, calcium, and potassium. Bees can use the proteins it provides as a protein supplement. Unlike other species of animals, bees are not affected by tannins. When bees are actively foraging across long distances, they rely on the carbohydrates in Sorghum to keep them going [12].

Brewer's yeast is a valuable ingredient for bee diets; it is rich in protein, which is critical for the growth and development of honeybees, especially for larvae and young bees. Similarly, soybean flour contains high levels of protein, essential for the production of bee brood (eggs, larvae, and pupae) and for the overall health of the bee. While bees do not digest fibre amount not exceeding 7% in their diet can be beneficial for gut health and overall colony function. Fibre acts as a prebiotic, providing a substrate for beneficial gut bacteria to thrive well. A healthy gut microbiome in honeybees is crucial for proper digestion, absorption, and nutrient utilization [13].

Chickpea (Cicer arietinum) is a legume known for its high protein content and relatively low water requirement, making it suitable for growth in dry regions [14]. Chickpea flowers provide nectar and pollen, which are critical for honeybee nutrition as well flour which can be combined with other viable ingredients to make pollen patties. This crop is already grown in arid and semi-arid regions, meaning its availability as a forage source could be cost-effective and locally sustainable. Prosopis juliflora, a hardy invasive species in some parts of Kenya, particularly in the ASAL (Arid and Semi-Arid Lands) regions, has seed pods rich in carbohydrates and protein [15]. While often considered a menace due to its aggressive spread, the use of its pods as a bee feed offers a sustainable management option for the plant while supporting local apiculture. Prosopis pods could provide critical nutrients during periods when natural nectar sources are limited, potentially improving honeybee survival rates and honey production. With climate change and drought reducing the availability of natural forage, these locally available, drought-tolerant crops offer a viable solution for sustaining honeybee populations in these challenging environments [16].

Ash content represents the mineral content of the ingredients, which are essential for various physiological functions in honeybees, including enzyme activation, nerve function, and overall health [17]. Minerals act as co-factors for enzymes involved in various biochemical reactions for example magnesium is responsible for activation of enzymes involved in energy metabolism and protein synthesis. Potassium and Calcium are essential for nerve function in honeybees whereas magnesium and potassium are involved in muscle contraction and relaxation hence assisting honeybees during flight, foraging and other related activities [18].

Incorporating natural pollen and other high-protein foods into the recipe is crucial for satisfying the honeybees' nutritional needs, even though it cannot completely substitute the variety of nutrients present in pollen. This experiment was conducted to assess the nutritive value of chickpea (*Cicer arietinum* L.), Prosopis pods (*Prosopis juliflora*), and soybean (*Glycine max*)-based bee diets and their effects on larval development, maintenance of colony strength, and honey production. Chemical analyses determined the dry matter, ash, crude fibre, crude protein, and fat content of the diets using the AOAC [19] standard procedures.

Limitations of the study

The study focused on proximate composition (crude protein, fat, ash, crude fibre) without assessing other important micronutrients such as vitamins, minerals, and amino acid profiles, which are essential for bee health and productivity. A broader nutrient analysis, including micronutrients and bioactive compounds, should be conducted to determine the complete nutritional value of alternative feed sources and their impact on bee health and longevity. The study relied on *Prosopis juliflora* pods harvested from a specific location (KALRO, ABIRI Centre), which may not fully represent the variability in nutritional quality of *Prosopis* from different regions. A multi-location study should be conducted to assess the variability in nutrient composition of *Prosopis juliflora* pods from different regions, as environmental factors can influence pod quality.

Material and Methods

Study Site

Field experiment was carried out from March to August 2023 at the Dryland Research Training and Ecotourism Centre (DRTEC), Chemeron Marigat, Baringo County, Kenya. It is an interdisciplinary research and coordination centre for Egerton University, which is 1080 m above sea level 0°28'10.1"N 35°58'59.79"E. It receives 700-950 mm rainfall per year with peaks in April and July/August, but is generally very erratic. The annual mean temperature is 23°C [19]. Feed ingredients were subjected to proximate analysis at Egerton University, Department of Animal Sciences Animal Nutrition laboratory. Egerton University is in Njoro sub-county, Nakuru County, 0°22'11.0 "S, 35°55'58.0 "E, 1,800m above sea level with an average temperature of between 17° C and 22° C. The annual rainfall is 1,200±100 mm [20].

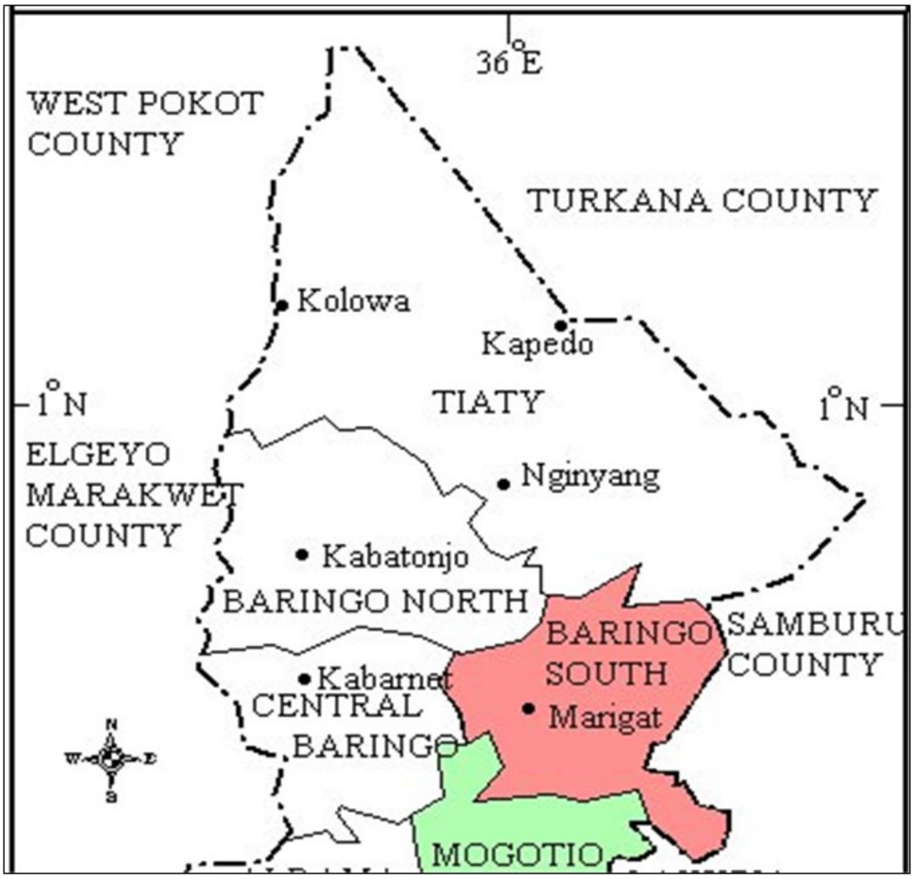

Figure 2.1: Map showing the Study Site

Source: Survey of Kenya, 2022

Collection of *Prosopis juliflora* Pods and Preparation of Experimental Diets

Mature Prosopis pods were harvested by plucking from the branches at the Kenya Agriculture and Livestock Research Organisation (KALRO), ABIRI Centre. The Centre is located in Marigat Sub-County, Baringo County, Kenya (0°28'10.1"N, 35°58'59.79"E). The Centre is located in Marigat Sub-County, Baringo County, Kenya. It receives 700-950 mm of rainfall per year, with peaks in April and July/August, but is generally very erratic. The annual mean temperature is 23°C [19]. They were sorted, dried and then ground to pass through a 1mm screen. After grinding, the pod flour was packaged in airtight glass jars to protect it from external contaminants, stored in a cool, dark, and dry place to avoid exposure to sunlight and heat, which can intensify nutrient degradation. This storage was meant to ensure preservation of nutrients, extend shelf life and to avoid spoilage.

Preparation of Experimental Diets

There were three experimental diets with three replicates each: T1= 30% soybean+45% sorghum flour+10% skimmed milk + 15% brewer's yeast T2= 30% chickpea+25% sorghum flour+ 5% skimmed milk + 40% brewer's yeast T3= 20% Prosopis pod flour +30% sorghum flour+ 5% skimmed milk + 45% brewer's yeast (Table 2.1). This study relied on guidelines and best practices developed by experts in the honeybee nutrition field.

Table 2. 1: Proportion of Different Ingredients in the Diets

Component	T1(30% Soybean)	T2(30% Chickpea)	T3(20% Prosopis pod flour)
Soybean (%)	30	0	0
Chickpea (%)	0	30	0
Prosopis pod flour (%)	0	0	20
Sorghum flour (%)	45	25	30
Skimmed milk (%)	10	5	5
Brewer's yeast (%)	15	40	45
Total (%)	100	100	100

Proximate Analysis

Dry matter (DM) was determined by drying in a hot air oven at 105°C for 24 h following standard method 925.09 [11], Ash by burning the samples in a muffle furnace at 550°C for eight hours following standard method 923.03 [11], ether extract by the Soxhlet method (using ether) following standard methods 920.39 (AOAC, 2006). Total nitrogen for crude protein (N x 6.25) determination was obtained using the micro-Kjeldahl method following standard methods 920.87[11].

Statistical Analysis

The statistical model used was:

$$Yij = \mu + \tau i + \varepsilon ij$$

Where: Yij was the random variable representing the response to the treatment I observed. μ was the overall mean; τi was the (additive) effect of the i^{th} treatment, (i = 1, 2, 3, 4); εij was the random error for the i^{th} treatment.

Data Analysis

All variables were subjected to analysis of variance (ANOVA) in a completely randomized Design (CRD) utilizing the SAS 9.4 (SAS, 2009). Means were separated using the Duncan Multiple Range Test (DMRT) procedure at a 0.05 Significance level (SAS, 2009).

Results

The proximate analysis of the ingredients differed significantly ($p<0.05$), as shown in Table 2.2. Brewer's yeast recorded the highest DM with 950.0 gKg^{-1} DM, followed by Soybean flour with 948.7 gKg^{-1} DM, while Chickpea flour recorded the lowest with 913.6 gKg^{-1} DM. Soybean flour contained the highest with 437.0 gKg^{-1} DM, followed closely by Brewer's yeast with 420.8 gKg^{-1} DM. Chickpea flour had 208.6 gKg^{-1} DM, while Prosopis pods had 151.1 gKg^{-1} DM, and the lowest was Sorghum flour with 98.4 gKg^{-1} DM. Brewer's yeast had the highest ash content with 66.8 gKg^{-1} DM, with the lowest being Sorghum flour with 16.5 gKg^{-1} DM. Soybeans had the highest crude fiber with (147.3 gKg^{-1} DM). At the same time, Brewer's yeast recorded the lowest with 19.2 gKg^{-1} DM. The fat content varied significantly, with Soybean flour having the highest with 188.3 gKg^{-1} DM, while ground Prosopis pods had the lowest with 18.7 gKg^{-1} DM.

Table 2.2: Proximate Composition of the ingredients (gKg^{-1} DM)

Ingredient	DM	Ash	CP	CF	Fats
Soybean flour	948.7	45.8	437.0	147.3	188.3
Chickpea flour	913.6	26.0	208.6	142.6	45.1
Prosopis juliflora	943.6	59.0	151.1	138.9	18.7
Brewer's yeast	950.0	66.8	420.8	19.2	36.5
Sorghum flour	921.6	16.5	98.4	39.3	28.2

DM=Dry matter, CP=Crude protein, CF=Crude Fiber

Results from the proximate analysis displayed varying trends across the treatments, as tabulated in Table 2.3. There was a significant difference ($p<0.05$) between the CP of diet T3 (250.6 gKg^{-1} DM), while T1 and T2 had no significant difference. (263.4 gKg^{-1} DM and 261.7 gKg^{-1} DM) respectively. Similar trends were observed in Ash content, with T3 recording the highest (39.1 gKg^{-1} DM), while T1 and T2 had no significance and recorded 36.5 gKg^{-1} DM and 34.4 gKg^{-1} DM, respectively. There were no significant differences ($p>0.05$) in CF across all the treatments in this study. However, significant differences were witnessed in the fat content at ($p<0.05$), with T1 recording the highest with 73.4 gKg^{-1} DM, followed by T3 with 40.0 gKg^{-1} DM, while T2 had the least with 31.2 gKg^{-1} DM.

Table 2. 3: Chemical Composition of the Supplemental Diets (gKg^{-1} DM)

Treatment	CP	Ash	CF	Fats
T1	263.4 ± 0.38^{a}	36.5 ± 0.05^{b}	73.5 ± 0.21^{a}	73.4 ± 0.12^{a}
T2	261.7 ± 0.12^{b}	34.4 ± 0.11^{b}	76.5 ± 0.07^{a}	31.2 ± 0.07^{c}
T3	250.6 ± 0.55^{a}	39.1 ± 0.04^{a}	72.7 ± 0.05^{a}	40.0 ± 0.06^{b}
p-value	0.0272	0.0066	0.03036	<0.0001

[a, b, c,] means in the same column with different superscripts are significantly different at p<0.05 T1= 30% soybean+45% sorghum flour+10% skimmed milk + 15% brewer's yeast T2= 30%chickpea+25% sorghum flour+ 5% skimmed milk + 40% brewer's yeast T3= 20%ground Prosopis pods+30% sorghum flour+ 5% skimmed milk + 45% brewer's yeast.

Discussion

The proximate composition of ingredients in this study showed significant variations in vital nutritional components, crude protein (CP), crude fiber (CF), Ash, and fats. Brewer's yeast had the highest CP content, similar to studies by Terefe [12]. Soybean flour also contained a high CP, concurring with the findings by Pope [13]. Brewer's yeast is a valuable ingredient for bee diets; it is rich in protein, which is critical for the growth and development of honeybees, especially for larvae and young bees. Similarly, soybean flour contains high levels of protein essential for the production of bee brood (eggs, larvae, and pupae) and for the overall health of the bee. While bees do not digest fiber, amount not exceeding 7% in their diet can be beneficial for gut health and overall colony function. Ash content represents the mineral content of the ingredients, which are essential for various physiological functions in honeybees, including enzyme activation, nerve function, and overall health.

A diverse range of minerals in the diet contributes to the overall well-being of the bee colony [14]. The presence of fats in the diet is essential for bees, especially during times when they need energy reserves, and is also necessary for the production of beeswax and other physiological processes [15]. The specific types of fats and their ratios in the diet can influence the overall health and productivity of the bee colony. Diet T1, which included 30% Soybean, 45% Sorghum flour, 10% Skimmed milk, and 15% Brewer’s yeast, showed a considerably higher crude protein (CP) content compared to treatments T2 and T3 in the supplemental diets. The finding is consistent with a previous study by Jach [16] that suggests the presence of specific ingredients can influence the amounts of CP in animal diets.

Ash content was most significant in T3, which had a more considerable amount of Brewer's yeast. This implies a possible correlation between the concentration of yeast and the mineral content, a phenomenon previously investigated by Delphine [17]. Brewer's yeast's high mineral content may be the cause of T3's greater ash level. Brewer's yeast was found to have a significant impact on ash content; adjusting its concentration in formulations to achieve desired mineral levels in the final product is crucial. There was considerable variance in the fat content of the diets, with T1 having the most significant quantities.

This aligns with the findings of Ricigliano [18], who emphasized the impact of ingredient ratios on the lipid composition of animal meals. The increased fat level in T1 can be due to the incorporation of Soybean and Brewer's yeast, both of which are recognized for their comparatively elevated fat content. This implies that bees consuming this diet T1 had access to more energy. They require this energy for various activities such as foraging, hive maintenance, and temperature regulation. The increased fat content may contribute to meeting these energy demands. These findings highlight the significance of carefully choosing ingredients and determining the appropriate ratios when developing bee diets to fulfill specific nutritional needs. Subsequent investigations could prioritize the determination of amino acids and minerals to augment the nutritional composition of bee feed, hence enhancing honeybee performance and health.

Conclusion and Recommendation

This study found that Brewer's yeast and soybean flour are notably good supplements in the bee diet due to their high protein content. Additionally, combining different protein sources in the diet can help ensure a more complete amino acid profile. Overly, T1= 30% soybean+45% sorghum flour+10% skimmed milk + 15% brewer’s yeast was the best ranked diet. This study recommends making use of high-protein sources, such as Brewer's yeast and Soybean flour combined with sorghum and skimmed milk to come up with high quality pollen supplement for honeybees.

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