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Assessing the Viability of Incorporating Sunflower Meal and Roselle Seed Meal as Unconventional Protein Sources in the Diets of Japanese Quails on Growth Performance, Carcass traits, Serum Metabolites, Gene Expression, and Economic Efficiency

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Abstract

THIS study aimed to assess the effects of replacing soybean meal (SBM) in the diet of growing Japanese quails with sunflower meal (SFM) and roselle seed meal (RSM) at levels (5 and 10%). The analysis covered performance, carcass trait, some blood serum metabolites, gene expression, and economic efficiency measures. When comparing the quails in the experimental groups to those in the control group, there was no discernible difference in terms of their ultimate body weight or feed conversion ratio. However, body weight gain of growing quails fed 10% SFM or RSM showed higher body weight gain than other treatment group. The non-significant differences indicate that these alternative protein sources were at least as effective as soybean meal in supporting quail performance in the studied conditions. Blood serum parameters revealed that no statistically significant differences in total protein levels, HDL, and lipid peroxidation among the control group and the experimental groups. Nevertheless, it is significant that the cholesterol levels in all experimental groups were greater than those in the control group. The expression profiles of *GH*, *IGF-I*, *PGAM2*, *IFN-α*, *IL-2*, *IL-4*, *IL-6*, *IL8*, *SOD*, and *GPX* were considerably upregulated in the 10% SFM or RSM groups compared to other groups. MSTN, however, produced the opposite tendency. Significant economic efficiency measures were observed with the best results showing with a group feeding diet incorporating 10% SFM followed by 10% RSM incorporation. In conclusion, the utilization of SFM and RSM, especially at the 10% inclusion level instead of SBM, led to enhanced growth performance, improved expression of growth-related, immune, and antioxidant markers and improved economic efficiency in Japanese quails.

Keywords: Quail; Performance, Sunflower meal, Roselle seed meal; Gene expression, Economic evaluation.

Introduction

The primary barrier to the longstanding sustainability of intensive quail production is the high cost of feed, which makes up around 70% of all production expenses [1]. The greatest way to reduce cost is to design diet formulas with alternative, locally sourced ingredients, which will lower feed costs. Protein sources are becoming more difficult to obtain all around the world. Consequently, finding substitute protein sources is necessary [2]. One of the most often used forms of protein in poultry diets is soybean meal (SBM). Nutritionists must use the ingredients that are easily available to create an economical, balanced diet [3]. Lately, the escalating expense of (SBM), with prices reaching 18 Egyptian pounds per kilogram, has emerged as a pivotal factor affecting the economic viability of the poultry meat industry, especially in economically challenged countries [4].

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In this context, research has been conducted to find new, different, and economically feasible plant protein bases to substitute a part of the SBM in the diets of chickens, especially if these different ingredients are readily available locally.

Sunflower meal is a potential, cost-effective protein source that can be utilized to partially substitute soybean meal (SBM) in the diets of poultry, a cheap by-product of the agro-industry meal (9L/E for each kilo of SFM) [5]. Its exceptional adaptability to a wide variety of soil and climate conditions accounts for its widespread availability worldwide. It is regarded as a protein concentrate, offering an abundant source of plant-based protein, alongside its notable content of vegetable oil. Furthermore, its high lipid content, comprising 65.42%, positions it as a valuable source of energy in feed formulations [6,7]. Because of the anti-nutritional factor, animal performance should be considered as the genuine indicator of its nutritional content; SFM commonly has fewer antinutritional elements than other oilseed meals [8]. Sunflower is rich in linoleic acid as well as, naturally occurring antioxidants, and a readily available supply of B vitamins, calcium, and phosphorous [9,10].

Roselle seed meal shows promise as a prospective alternative protein ingredient in poultry diets. It is not commonly used for human consumption, making it an economical and readily available resource. This characteristic also reduces the risk of potential scarcity [11]. RSM stands out as an outstanding alternate to soybean meal in broiler diets, considering various aspects such as economic feasibility, accessibility, nutritional value, and the bioavailability of its nutrients, particularly in terms of digestible protein and digestible energy [12]. Roselle seed contains 22% crude protein, 89% Dry matter, 20% crude fiber, 21% lipid, and 6.4% ash content & high anthocyanin content (Delphindin in -3 sambubioside and cyanidin-3 – sambubioside) is beneficial as a possible source of antioxidants and a colorant [13,14]**.** Studies have indicated that RSM is rich in essential amino acids, including lysine, arginine, leucine, phenylalanine, and glutamic acid [15]. Processing to inactivate anti-nutritional elements will be necessary for non-ruminant animals to use RSM effectively [16].

The chief objective of this research was to examine the impacts of incorporating SFM and RSM at different levels (5% and 10%) into the diets of growing Japanese quails. The study aimed to assess how these dietary changes influenced various aspects, including growth performance, carcass characteristics, serum metabolites, gene expression patterns, and economic efficiency.

Material and Methods

Experimental design, housing, and diets

The Mansoura University Animal Care and Use Committee (MU-ACUC) has approved the experimental protocol with code number VM.R.22.11.22. The trial was carried out at Mansoura University's Faculty of Veterinary Medicine's Laboratory of Animal Nutrition & Clinical Nutrition.

A total of 400, one-week-old mixed-sex Japanese quails were divided into 5 identical treatments, each consisting of 8 duplicates of 10 quails. The Japanese quails were housed in (50 x 50 x 40 cm) plastic wire floor pens for four weeks. At the commencement of the experiment, the temperature in the pens was maintained at 34°Cat floor level. Over the following weeks, the temperature was progressively reduced by 2°C per week until it reached a range of 24–26°C. The quails had easy access to water and feed thanks to the manual drinkers and self-feeders installed in each cage. Throughout the experiment, artificial lighting was present for 24 hours each day. The experiment's design was completely random. Five distinct experimental diets were formulated, each containing an identical level of crude protein (24%) and 2900 kcal/kg of metabolizable energy. Each diet came in mash form and was isonitrogenous and isocaloric. Furthermore, each of these experimental diets was meticulously formulated to fulfill all the essential nutrient requirements essential for the growth of Japanese quails as specified in the NRC [17] (Table 1). The sunflower meal and roselle seed meal utilized in this experiment were procured from the Dakahlia Poultry Company located in Mit Ghamr. Table 2 provides a thorough breakdown of the nutrients found in these components, which were employed in place of soybean meal in this investigation. Within the experimental setup, Japanese quails in the control group were provided with a standard base control diet. In contrast, the other four experimental treatments included SFM and RSM as alternative sources of protein at levels of 5% and 10% [18,19].

Growth performance parameters

In the study, growth performance was assessed through several parameters:

Daily Feed Intake: The daily amount of feed consumed by the quails was measured and recorded throughout the experimental period.

Weekly Body Weight: The body weight of the quails was measured and documented on a weekly basis.

Feed Conversion Ratio (FCR): FCR was computed to assess feed utilization efficiency. It represents the amount of feed required to produce a unit of body weight gain. A lower FCR indicates more efficient feed conversion.

Body Weight Gain (BWG): This parameter quantifies the increase in body weight of the quails over a specific time period, typically a week.

Samples collection

Carcass traits

At the conclusion of the 35-day trial, 24 quails per treatment—three from each replicate—were chosen at random, weighed, and slaughtered by having the neck cut off with a sharp knife. The weight of the whole carcass, dressed carcass, breast, thigh, visceral and lymphoid organs was recorded and evaluated as a percentage related to living body weight. 50 mg samples of the liver, muscle, and spleen were obtained immediately after the animal was slaughtered, snap-frozen in liquid nitrogen, and kept at 80 °C until they were utilized for RNA isolation.

Serum biochemical parameters

The blood samples from the same Japanese quails used for carcass traits were obtained before slaughter, and they were stored at -20°C for further analysis after being centrifuged for 10 minutes at 3000 rpm. The serum total protein (TP) and albumin (Alb) were measured using (Stanbio laboratory) USA kits [20]. Triglycerides, cholesterol, and high-density lipoprotein (HDL) cholesterol were measured using kits made by (Spinreact) Spain [21]. Lipid peroxidation (MDA) level determination using an enzymatic colorimetric technique.

RNA extraction and Reverse transcription

Total RNA was isolated from quail muscle, liver, and spleen tissues with Trizol reagent (Direct-zolTM RNA MiniPrep, catalogue No. R2050), in accordance with the manufacturer's directives. Quantity and purity were evaluated with a Nanodrop (UV-Vis spectrophotometer Q5000/USA), and integrity was valued using gel electrophoresis. The manufacturing approach (SensiFastTM cDNA synthesis kit, Bioline, catalogue No. Bio-65053) was used to synthesize the cDNA in each sample. The reaction mixture consisted of 20 µl of total RNA up to 1 µg, 4 µl of 5x Trans Amp buffer, 1 µl of reverse transcriptase, and 20 µl of DNase-free water.

Using a thermal cycler, the reaction mixture was subjected to the following protocol: 10 minutes of primer annealing at 25 °C, 15 minutes of reverse transcription at 42 °C, and 5 minutes of inactivation at 85 °C. The samples were retained in a 4 °C environment.

Quantitative Real-Time PCR

The mRNA levels of growth performance, immune system, and antioxidant markers were relatively measured using the SYBR Green PCR Master Mix and real-time PCR (2x SensiFastTM SYBR, Bioline, catlog No. Bio-98002). Table 3 showed the primer sequences that were displayed. As an internal control, the housekeeping gene *β-actin* was employed.

0.8 µl of each primer, 5.4 µl of d.d. water, 10 µl of 2x SensiFast SYBR, 3 µl of cDNA, and 10 µl of 2x SensiFast SYBR made up the reaction mix, which was conducted in a complete capacity of 20 µl. The cycling settings for the PCR were as follows: two minutes at 95 °C, forty cycles at 94 °C for denaturation, twenty seconds for annealing at the temperature specified in Table 3, and twenty seconds for an extension at 72 °C. Following the amplification stage, the specificity of the PCR product was verified via a melting curve study. The comparative expression of each sample's gene for *β-actin* in relation to a control gene was determined using the $2^{-\Delta\Delta Ct}$ technique [22].

Economic efficiency studied parameters

At the experiment's end (4th week), quails were sold and we collect economic data for analysis. The cost of composited diets was considered in Kg and ton, depending on the market prices of feed components at experiment period, cost of feed intake/quail and /group, feed cost /kg quail meat gained (cost of feed intake/ body weight gain), feed cost savings per kg meat gained (cost of feed/kg meat of controlled group – feed cost/ kg meat gain of a tested group) [23].

Costs of production

It was calculated for each quail and they are divided into Total variable costs (TVC), Total fixed costs (TFC), and Total costs (TC) [24,25].

TVC= cost of quail purchased (5LE) + feed cost/ quail according to Atallah [26].

TFC is the total of the following: labour, litter, water and electricity, building and equipment rent, miscellaneous and transportation costs, and overall veterinary management (drugs and vaccines) [27,28].

TC=TVC+TFC according to Sallam et al [29].

Total returns (TR): according to Atallah [30] and El-Sheikh et al [31].

Total returns (TR)/quail = Litter sale/quail + quail sale

Litter sale /quail = total litter sale /number of quails

(Quail sale at the time of experiment =15LE/quail)

Net Profit (NP): calculated/quail as previously [24,30] by this equation: NP = Total returns (TR) – Total costs (TC).

Economic efficiency measurements,

Collective and partial efficiency measures were estimated [30,32]. They consist of percentages of feed cost to TC (feed cost / TC), feed costs to TVC (feed costs/TVC), total return to total costs (TR/TC), the total cost to the total return (TC/TR), net Profit to Total Cost (NP/TC) and net profit to the total return (NP/T).

Statistical Analysis

The SPSS 20 software was used to conduct the statistical analysis (SPSS Inc., Chicago, Illinois) to evaluate the effects of applying SFM& RSM on growth performance, carcass trait, serum metabolites, gene expression, and the economic effectiveness of developing Japanese quails. To analyze the collected data, one-way ANOVA and Duncan's multiple comparisons of the means were used. Standard deviations and averages were used to display the data. Differences between interventions were reflected significant when P<0.05.

Results

Growth performance

Table 4 shows the growth performance of the Japanese quails fed diets comprising SFM & RSM at levels (5& 10%). There were no discernible variations in the end weight or body weight growth between the experimental and control groups. Compared with the control group, the groups fed at a 10% level of either SFM or RSM showed noticeably increased BWG. The diets that converted feed to body weight increase most effectively were the 10% SFM and RSM groups, as evidenced by their lowest FCR values.

Carcass traits

The findings related to carcass characteristics can be found in Table 5. The results indicate that incorporating 5% SFM into the diet had a positive impact on the dressed carcass percentage. In contrast, 5% RSM had a negative impact, reducing the dressed carcass percentage. However, neither 10% SFM nor 10% RSM had a significant effect on dressing percentage compared to the control. The various dealings had no discernible effects on the percentage of dressed carcasses, liver, breast, or thigh. The percentage weights of immunological organs were unaffected by dietary treatment.

Blood serum parameters

Table 6 displays the serum parameter results of quails fed varying amounts of SFM & RSM in place of SBM. All experimental groups showed similar levels of lipid peroxidation, high-density lipoprotein, and total protein. The albumin concentration was significantly (P < 0.05) higher with quails fed 10% SFM & (5& 10%) RSM in contrast to the remaining study groups. The control group had a much lower total cholesterol level than the other groups. The triglyceride level at 5% RSM inclusion was significantly higher than the experimental groups. LDL levels were higher in the group fed RSM (5& 10%) than in other treatment groups.

Gene expression profile of growth performance, immune and antioxidant markers

For 4 weeks growth period, supplementing sunflower meal or roselle seed meal may modify the expression profiles of the genes involved in growth performance (*GH*, *IGF-I*, *MSTN*, and *PGAM2*), immune (*IFN-α*, *IL-2*, *IL-4*, *IL-6*, and *IL8*) and antioxidant (*SOD* and *GPX*). The expression profiles of *GH*, *IGF-I*, PGAM2, *IFN-α*, *IL-2*, *IL-4*, *IL-6*, *IL8*, *SOD*, and *GPX* were considerably unregulated in the 10% sunflower meal or 10% roselle seed meal groups compared to the 5% and soybean meal groups. MSTN, however, produced the opposite tendency (Figures 1, 2 & 3).

Economic efficiency

Using sunflower meal and roselle seed meal in place of soybean meal by 5% or 10% considerably (p<0.001) reduced the cost of feed per kilogram, per ton of feed, per group, or quail, and a kilogram of quail meat gained Table 7. All of these were found to be higher in the control group, as well as TVC and TC/quail. SFM 10% had the lowest feed cost per quail, followed by RSM 10%. Meanwhile, RSM 10% had the lowest feed cost/kg quail meat gained, followed by SFM 10%. On the other hand, SFM 10% had the highest feed cost saving /kg quail meat gained. The TVC and TC were the lowest values in the SFM 10% group, followed by the RSM 10% group, whereas the highest NP and also the best in all economic efficiency measures were in group SFM 10% followed by RSM 10%.

Discussion

Based on the data collected for this investigation, the FBW of the quail did not show any statistically noteworthy variances between the experimental and control groups. Our findings align with the results achieved by Alagawany et al. [33] who found that using SFM at different levels (25%, 50%, 75%) as a replacement for SBM in broiler diets enhances broiler growth. In contrast, a different investigation found a negative conclusion on the performance of broilers by the addition of SFM to diet [34]. Likewise, it has been noted that the addition of 15% SFM in the diets of hens did not have an impact on weight gain [35]. Moreover, roasted roselle seed meal can be used to replace

50% groundnut cake without any harmful effects on broiler chicken [36]. The findings of this experiment are also consistent with those cited by Ashom et al [37].

RSM has high nutritional value, health-promoting nutrients like gosypetine and functional flavonoids, as well as nutrients like protein, oil, dietary fibre, glutamic acid, lysine, arginine, leucine, and phenylalanine [38]. The incorporating moderate amounts of various fiber sources into the diet promotes the growth of the digestive system and stimulates the production of bile acids, enzymes, and hydrochloric acid (HCl) [39]. These modifications may lead to enhancements in nutrient absorption, growth efficiency, gastrointestinal tract health, and finally, animal comfort [40].

The results obtained concerning quail's performance may be related to heat treatment's improved nutritive value by the reduction in anti-nutritional factors and the enhancement of protein digestibility, which could potentially lead to improved performance [41]. The high FBW & BWG in the group received 10% SFM & RSM (230, 236 g/ bird) than others (224, 220 & 221 g / bird) in control and other experimental groups may be associated with various plant-related factors. These factors could include the type of plant extracts utilized, their dosage, the presence of volatile fatty acids, the rate of active substances, and the potential interactions between these elements. Furthermore, improving performance required the availability of vitamin C in RSM. Furthermore, the incorporation of citric acid, tartaric acid, malic acid, and tartaric acid is recognized for its capacity to foster a healthier stomach flora which in turn results in increased absorption of nutrients [42]. Moreover, the significant improvement in the feed-to-gain ratio observed with SFM may be attributed to a synergistic interaction among nutrients. With the rise in the percentage of sunflower meal in the diet so did the inclusion of oil, effectively meeting the birds' energy necessities and enhancing the diet's digestibility [43]. Furthermore, the utilization of SFM resulted in an increase in apparent metabolizable energy, potentially contributing to the enhanced feed-to-gain ratio**.**In accordance with our findings, Muthui et al. [44] also observed that the utilization of unconventional feed materials enhances the growth rate of pigs. Furthermore, El Dakroury [45] discovered that Moringa Oleifera Leaves Powder can serve as a natural, efficient, and secure substitute for growth-promoting antibiotics in broiler chickens.

The best record of feed conversion ratio (FCR) was reported at level (10%) SFM & RSM (3.29). FCR is an important factor that reflects the nutritional assessment of the feed and the effectiveness of utilization. Our results align with the outcomes observed in broiler studies on feeding different levels from RSM and found better FCR at level 7.5% [46]. Sangsoponjit et al. [47] reported similar outcomes, observing that adding 8% sunflower meal raised body weight and improved FCR. Therefore, it can be inferred that using (SFM & RSM) in quail's diets maintains performance & improved FCR, especially at level 10%.

Another important finding of the current experiment was that the inclusion level of SFM & RSM in the diets did not affect the carcass traits of quails. This finding in the group of SFM were reliable with the verdicts of Tuzun et al. [48] who noted that the utilization of SFM in the diets of growing quails at a 10% level did not have any discernible influence on carcass characteristics and relative organ weights. Furthermore, sunflower meals in broiler diets at different levels (0, 15, and 30%) found no noticeable effect on carcass attributes [49]. Our results in the group of RSM were in line with the previous reports [19,36] that utilized various levels of RSM in broiler did not influence carcass weight and internal organs.

Serum biochemical parameters are an indication of the overall health, nutritional status, management practices, exposure to external factors, and the productive performance of poultry, as well as providing insights into the presence of metabolic diseases [50]. No significant variations in total protein were seen between all experimental groups. Additionally, quails fed 10% SFM & (5& 10%) RSM had considerably greater albumin concentrations ($P < 0.05$) than other experimental groups. Greater dosages of RSM and the greater protein quality of SFM are responsible for these outcomes [51]. Albumin and total serum protein are indicators of the liver's biochemical production of plasma proteins. In a conclusion, the liver's functionality was supported by the level of albumin, and RSM improved the health of broiler chickens [6]. There was a detectable rise in the level of cholesterol between each experimental group and the control one. This increase in cholesterol levels might be due to the supplementation of SFM & RSM may enhance the fat content in the bird [11].Moreover; further investigations are needed to evaluate fat content in SFM & RSM. Similar to our findings, using 250 g/kg of SFM in the diets of broiler chicks harmed blood parameters [52]. In addition**,** the highest results of triglyceride & cholesterol were found by chicks fed a diet supplemented with 50% SFM [6].

In this experiment, 10% supplemented SFM or RSM groups significantly up-regulated the expression profile of growth, immunological, and antioxidant markers compared to 5% supplemented and control groups. This study is the first that we are aware of that looks at the effects of SFM & RSM supplementation on the gene expression outline, growth efficiency, immune system, and antioxidant indicators in quail. Supplementing nutrition is a successful method for boosting the immune system and enhancing avian health [53]. According to the concentration in the broiler feed, the effects of supplementing with aromatic plant extracts on growth, immunology, and antioxidant status varied noticeably [54]. The higher levels of calcium, total phosphorus, gross energy, fatty acids, cystine and arginine, and methionine content in SFM compared to SBM may be the cause of the change in the expression configuration of growth, immune and antioxidant markers observed after supplementing quail with SFM 10% [2].

Because they prevent metabolic problems including diabetes and cardiovascular disease, unsaturated fatty acids (UFAS) are crucial for both animal and human diets [55]. Sunflower seeds are also incredibly rich in α-tocopherols, which are strong antioxidants. Although they are more common in soybeans, p- and y-tocopherols are more difficult to heat inactivate than α-tocopherols [56]. Sunflower is thought to contain more vitamin E which reduces the production of free radicals [57]. Vitamin E may be able to alleviate oxidative stress by preventing the oxidation of UFAs, altering eicosanoid metabolism, and modulating transcription factors that boost avian immunity [58]. Additionally, arginine content in SFM influences immunological responses because it participates in the production of polyamine and proline, which respectively, promote lymphocyte proliferation and repair injured tissues [59]. Additionally, arginine promotes tissue healing by triggering vasodilatation and using the no-generation pathway to regulate the immune system [60]. According to the aforementioned studies, SFM may improve the gene expression of growth, immunological, and antioxidant indicators.

In the same respect for RSM, our findings showed that 10% of RSM-supplemented quails had higher levels of mRNA for growth, immune, and antioxidant markers than the control group. The antifungal, antibacterial, antiviral, antioxidant, and anti-lipidemic belongings of aromatic plant extracts are noteworthy [61]. In broiler feeding, aromatic herbs also accelerate digestion and boost appetite [62]. Additionally, some researchers have discovered that volatile oils in quails and broilers have an effect that promotes growth [63-66]. Roselle seed meal is nutrient-dense, being high in vitamin C, polyphenols, carotenoids, certain minerals, essential oils, and good fats [38,67]. Additionally, because of the high concentration of phenolic chemicals, it can act as a natural antioxidant. According to Blount et al. [68] carotenoids are crucial for the immune system and antioxidant activity. RSM also contains significant amounts of tocopherol, a precursor of vitamin E, and vitamin C [38]. Therefore, it may be assumed that the active components of RSM were likely responsible for this enhancement in the transcription of growth, immunological, and antioxidant markers.

Regardless of whether SFM or RSM is fed at different levels, the overall fixed costs of production are not affected, since all groups are expected to pay for labor, litter, all veterinary managing costs (drugs and vaccines), water and electricity, rent for buildings and equipment, miscellaneous and transportation costs by the same amount based on [69,70]. In response to differences in SFM, RSM, and SBM prices, contrary to SBM, SFM, and RSM are cheap by-products when replacing SBM with SFM or RSM resulted in a lower feed kg cost, which in turn resulted in a lower feed ton price. We agree with Kamel et al. [71] who found that incorporating non-conventional feed resources into feed decreases the cost of one ton of feed at all intensities. The results of this study are also consistent with those cited by previous studies [72,73] where the low cost of non-conventional feed resources resulted in a decline in diet costs. Accordingly, the feed cost of the control group was the highest because feed kg was more expensive as well as more feed intake/quail.

As a consequence of the decrease in feed cost and the amount of feed/quail in SFM and RSM groups, the TVC and TC of these groups also decreased since feed costs constitute the largest input cost about 60-70% of production costs in most production farms [74]. This is in agreement with the outcomes issued by El-Sheikh et al. [31] and Alqaisi et al [75] who demonstrated that the inclusion of non-traditional feed resources in the diet decreases TC of production. You saved more money /kg meat gained when SFM or RSM was incorporated into diets since feed costs /kg meat gained were lowered. Consequently, you fed less and gained more weight, similar results have been reported when using other non-conventional feed sources [76,77].

Regarding TR similarity, the number of quails and their price on the market, as well as how much litters sold, were the same. There was a difference in NP between groups because their TCs are different and NP=TR-TC. When SFM 10% and RSM 10% were used, the profits were higher, these results come in line with other researchers' findings that using other non-conventional feed resources in diet produced better results than a control group [77,78]. Based on economic efficacy indicators such as feed cost/ TC, TR/TC, NP/TC, TC/TR, and NP/TR, when SFM or RSM are incorporated into the diet resulted in better results than the control group as they had lower feed costs and TC with higher TR and NP. It is consistent with the outcomes of Kamel et al. [79] and Attia et al. [80] who found that adding non-conventional feed resources to quail diets increased their efficiency.

In the same way, sunflower meal improves the production efficiency measures of quails [81]. It is recommended economically to substitute either sunflower meal or roselle seed meal instead of SBM up to 10%, but the most efficient percent is SFM 10% followed by RSM 10%.

Conclusion

The results of this investigation indicate that SFM and RSM as by-products exhibit good nutritional quality, leading to performance levels similar to those achieved with the control diet. This suggests that SFM and RSM could serve as economically viable and novel sources of protein fortification for a range of food products and hold promise as potential food ingredients. However, it's important to note that the usage of SFM and RSM in the quail diet seemed to affect some blood indicators, particularly triglycerides and cholesterol. These blood parameters showed unfavorable changes, possibly indicating that further research and dietary adjustments may be needed to mitigate these effects and fully harness the potential of SFM and RSM as substitute protein sources in poultry nutrition. Additionally, evaluating the long-term effects and potential ways to counteract these negative impacts could be essential in promoting the utilization of SFM and RSM in quail diets.

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Animal Welfare

The authors confirm that they have adhered to ARRIVE Guidelines to protect animals used for scientific purposes.

TABLE 1. Ingredients and calculated composition of control & experimental diets.

	Experimental diets*				
	Control	**SFM**		**RSM**	
Ingredients %		**5%**	**10%**	**5%**	**10%**
Yellow corn	51.3	50.8	50.7	49.7	48
Soybean meal	27	22.3	20	22.9	23.6
Corn gluten	10	11	11	11.4	10
Wheat bran	7.8	7.1	4.6	7.7	5.1
Mixed Oil	0.7	0.5	0.4	0.1	0.1
SFM	-	5	10	-	-
RSM	-	-	-	5	10
Limestone	1.5	1.5	1.5	1.5	1.5
Dica. Phosph.	0.6	0.6	0.6	0.6	0.6
Min. vit. Premix*	0.3	0.3	0.3	0.3	0.3
Common salt	0.3	0.3	0.3	0.3	0.3
DL.Methionine	0.1	0.1	0.10	0.1	0.1
DL. Lysine	0.4	0.5	0.5	0.4	0.4
Calculated value					
Crude protein %	24	23.9	23.9	24	24
ME (kcal/kg)	2901.8	2901.8	2901.9	2902	2901
Calcium %	0.79	0.80	0.81	0.80	0.82
Available phosphorus %	0.50	0.48	0.47	0.5	0.51
Calculated amino acids					
Methionine %	0.54	0.56	0.57	0.56	0.55
Lysine %	1.33	1.35	1.34	1.3	1.36
Tryptophan%	0.28	0.27	0.26	0.27	0.27
Threonine	0.85	0.86	0.88	0.85	0.87
Arginine	1.3	1.31	1.35	1.28	1.3
Valine	1.1	1.1	1.14	1.1	1.1

***Minerals & vitamins per kg of diet** Vitamin A, 16,500 IU; vitamin D3, 750 IU; vitamin E, 12 IU; vitamin K, 2 mg; vitamin B1, 1.2 mg; vitamin B2 10mg; vitamin B6, 2.4 mg; vitamin B12, 12 µg; niacin, 18 mg; pantothenic acid, 12 mg; Mn,190 mg as manganese sulfate; Zn, 72 mg as zinc oxide; Fe, 380 mg as ferrous sulfate; copper, 13 mg as copper sulfate; iodine, 0.4 mg as potassium iodide.

TABLE 2. Analyzed composition of sunflower meal & roselle seed meal used in experimental diets.

Items	Sunflower meal	Roselle seed meal
Crude protein%	28	21.35
Metabolized energy (kcal/kg)	2260	2876
CF%	13	13.7
Ash%	7.1	9.4
Lipid%	1.9	4.1

TABLE 3. Sequence, accession number, annealing temperature, and PCR product size of oligonucleotide primers used in real-time PCR for growth, immunological, and antioxidant genes.

Gene	Isolation source	Primer	Product length (bp)	Annealing temperature (°C)	Accession number
IFN-α	Spleen	F: CCTTGCTCCTTCAACGACA R: CGCTGAGGATACTGAAGAGGT	100	58	AB154298
IL-6	Spleen	F: CAACCTCAACCTGCCCAA R: GGAGAGCTTCCTCAGGCATT′	85	60	AB5595724
IL-8	Spleen	F: CTGAGGTGCCAGTGCATTAG R: AGCACACCTCTCTTCCATCC	139	58	AB559573
IL-4	Spleen	F: GAGAGCATCCGGATAGTGAAG R: TTCGCATAAGAGCTGGGTTC	168	62	AB559571
IL-2	Spleen	F: GTGCAAAGTACTGATCTTCGCC R: CTTGGTGTGTAGAGCTCGAGATG	195	60	AY613440.1
GH	Liver	F: GCTGCCGAGACATACAAAGAG R: GAGCTGGGATGGTTTCTGAG	109	62	FJ458436
IGF-I	Liver	F: CACCTAAATCTGCACGCT R: CTTGTGGATGGCATGATCT	140	60	FJ977570.1
SOD	Liver	F: TGGACCTCGTTTAGCTTGTG R: ACACGGAAGAGCAAGTACAGR	126	62	NM_205064.1
GPX	Liver	F: TTGTAAACATCAGGGGCAAA R: TGGGCCAAGATCTTTCTGTAA	140	58	NM_001163245.1
MSTN	Muscle	F: AGCACCTAACATTAGCAGGGACGT R: TTCACATCAATACTCTGCCAGATACC	422	60	XM_015867858.2
β-Actin		F: CTGGCACCTAGCACAATGAA R: CTGCTTGCTGATCCACATCT	123	55	AF199488

IFN-α = Interferon alpha; IL-6= Interleukin-6; IL-8= Interleukin-8; IL-4= Interleukin-6; IL-2= Interleukin-2; GH= Growth hormone; IGF-I= Insulin-like growth factor-1; SOD= Superoxide dismutase; GPX= Glutathione peroxidase; MSTN= Myostatin; and PGAM= phosphoglycerate mutase.

TABLE 4. Growth performance of growing Japanese Quails fed on a diet containing SFM & RSM by level (5& 10%) as a non-conventional protein supplement.

Parameters	Control	SFM5%	SFM10%	RSM5%	RSM10%	P value
Initial body weight (g/bird)	67.36±2.24	63.75±2.29	63.68±2.01	62.36±2.39	65.52±2.35	**Non significance**
Final body weight (g/ bird)	224±6.86	220±5.26	230±6.38	221±4..93	236±8.19	**Non significance**
BWG (g/ bird)	157.36±4.82 [b]	157.85±3.37 [b]	166.84±4.68 [a b]	158.68±2.61 [b]	170.52±6 [a]	**<0.05**
FCR (feed intake g/ body weight gain g)	3.68±0.10	3.48±0.06	3.29±0.08	3.45±0.05	3.29±0.10	**Non significance**

Data were presented as the mean of 24 quail ± SEM. Values with a different letter superscript in the same row indicate a significant difference between groups (P < 0.05).

TABLE 5. Effect of using SFM & RSM at levels (5 & 10%) on carcass characteristics & lymphoid organs % relative to the live weight of growing Japanese quails

Organ %	Control	SFM5%	SFM10%	RSM5%	RSM10%	P Value
Dressed carcass	69.6 ± 2.04 [a b]	71.17 ± 1.25 [a]	68.52±1.6 [a b]	65.69±1.45 [b]	67.48±1.06 [a b]	**<0.05**
Liver (%)	2.45 ± 0.12	2.46 ± 0. 38	2.37± 0.37	3.05 ± 0.046	3.09 ± 0.27	**0.1**
Breast (%)	45.69 ± 1.2	46.85 ± 1.06	44.7 ± 1.14	42.58 ± 0.71	44.50 ± 0.31	**0.07**
Thigh (%)	23.9 ± 1.05	24.31 ± 0.68	23.82 ± 0.55	23. 11 ± 0.68	23.13 ± 0.67	**0.1**
Lymphoid organs (%)						
Thymus	0.26 ± 0.03	0.3 ± 0.08	0.27 ± 0.04	0.42 ± 0.08	0.28 ± 0.03	**0.1**
Bursa	0.075 ± 0.008	0.095 ± 0.013	0.090 ± 0.01	0.107 ± 0.017	0.102± 0.023	**0.1**

Data were presented as the mean of 24 quail ± SEM. Values with a different letter superscript in the same row indicate a significant difference between groups (P < 0.05).

TABLE 6. Effects of using sunflower meal & roselle seed meal at levels (5& 10%) on blood serum parameters & lipid peroxidation of growing Japanese quails (day 42).

Parameters	Control	SFM5%	SFM10%	RSM5%	RSM10%	P value
Total protein	3.4 ± 0.15	3.9 ± 0.058	3.46 ± 0.088	3.6 ± 0.3	4.13 ± 0.185	0.07
Albumen	1.00 ± 0.173 [b]	1.40 ± 0.08 [b]	2.00 ± 0.058 [a]	1.76 ± 0.09 [a]	2.23 ± 0.12 [a]	0.001
Cholesterol	54.86 ± 15.89 [c]	141 ± 15.89 [a b]	151.3 ± 27.72 [a b]	193.3 ± 33.11 [a]	102.3 ± 10.9 [b]	0.01
Triglycerides	121.7± 10. 93 [b]	124 ± 10.26 [b]	111± 8. 5 [b]	166.7±17.64 [a]	104.3 ± 8.09 [b]	0.02
HDL	58.67 ± 9.4	55.33 ± 5.84	53. 67± 5.23	44.33 ± 1.68	57 ± 6.4	0.62
LDL	35.67±8.67 [bc]	27.33 ± 3.7 [c]	22 ± 1.5 [c]	57 ± 8.14 [b]	76.33 ± 1.8 [a b]	0.0002
MDA	36.67 ± 9.28	48.67± 4.7	48 ± 7.6	50.67± 2.6	41± 3.78	0.48

Values with a different letter superscript in the same row indicate a significant difference between groups (P < 0.05).

TABLE 7. Effect of using SFM & RSM at levels (5 & 10%) on economic efficiency measures of growing Japanese quails

Item	Control	SFM 5%	SFM 10%	RSM 5%	RSM 10%	SEM	*p*-value
Feed kg cost	15.59^{a}	15.44^{b}	15.27^{c}	15.25^{d}	15.12^{e}	0.038	0.001
Cost of one ton of feed LE/ton	15590^{a}	15440^{b}	15270^{c}	15250^{d}	15120^{e}	37.52	0.001
Total feed cost LE/group	169.43^{a}	159.88^{b}	157.54^{c}	158.20^{c}	158.00^{c}	1.028	0.001
Feed cost LE/quail	8.92^{a}	8.41^{b}	8.29^{c}	8.32^{c}	8.31^{c}	0.054	0.001
Feed cost LE/ kg quail meat gained	58.42^{a}	53.29^{b}	49.70^{d}	52.47^{c}	48.77^{e}	0.78	0.001
Feed cost saving LE/kg quail meat gained	0.00^{e}	5.13^{d}	8.72^{b}	5.95^{c}	9.65^{a}	0.78	0.001
TVC LE/quail	13.92^{a}	13.41^{b}	13.29^{e}	13.33^{c}	13.31^{d}	0.054	0.001
TFC LE/quail	1.95	1.95	1.95	1.95	1.95	0.00	-----
TC LE/quail	15.86^{a}	15.36^{b}	15.24^{e}	15.27^{c}	15.26^{d}	0.054	0.001
TR/quail	15.79	15.79	15.79	15.79	15.79	0.00	------
NP/quail	-0.08	0.43	0.55	0.52	0.53	0.054	------
Total feed cost/TC %	56.21^{a}	54.78^{b}	54.41^{e}	54.51^{c}	54.48^{d}	0.16	0.001
TC/TR %	100.48^{a}	97.29^{b}	96.51^{e}	96.73^{c}	96.67^{d}	0.34	0.001
TR/TC %	99.52^{e}	102.78^{d}	103.61^{a}	103.38^{c}	103.45^{b}	0.35	0.001
NP/TC %	-0.48^{e}	2.78^{d}	3.61^{a}	3.38^{c}	3.45^{b}	0.35	0.001
NP/TR %	-0.48^{e}	2.71^{d}	3.49^{a}	3.27^{c}	3.33^{b}	0.34	0.001

L.E: Egyptian Pound. The mean values with different superscript letters within the same row differ significantly at ($P < 0.001$). SEM - Standard Error of Mean, TC: Total costs, TVC: Total variable costs, TFC: Total fixed costs, TR: Total returns, NP: Net profit.

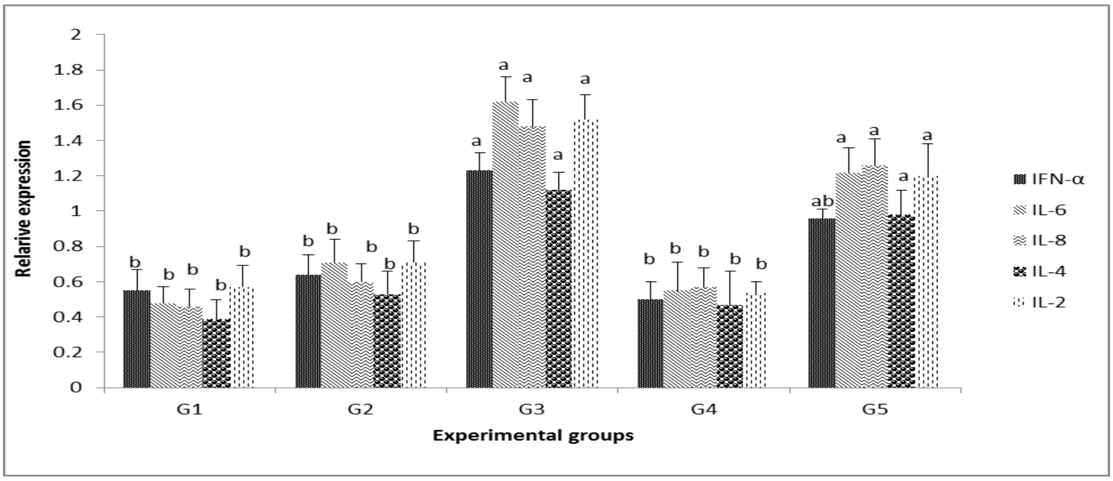

Fig. 1. Relative expression of immune markers in sunflower meal and roselle seed meals supplemented groups. G1= control group; G2= sunflower meal 5%; G3= sunflower meal 10%; G4= roselle seed meal 5%; and G5= roselle seed meal 10%. Small alphabetical letters show significance when (P<0.05).

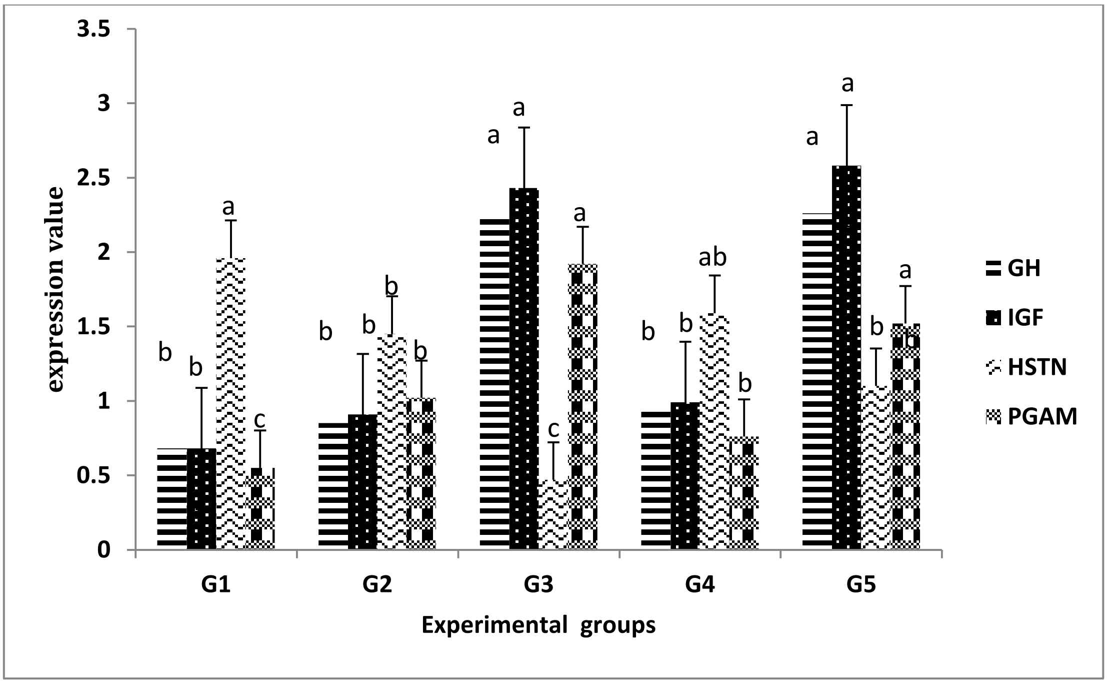

Fig. 2. Relative expression of growth markers in sunflower meal and roselle seed meals supplemented groups. G1= control group; G2= sunflower meal 5%; G3= sunflower meal 10%; G4= roselle seed meal 5%; and G5= roselle seed meal 10%. Small alphabetical letters show significance when (P<0.05).

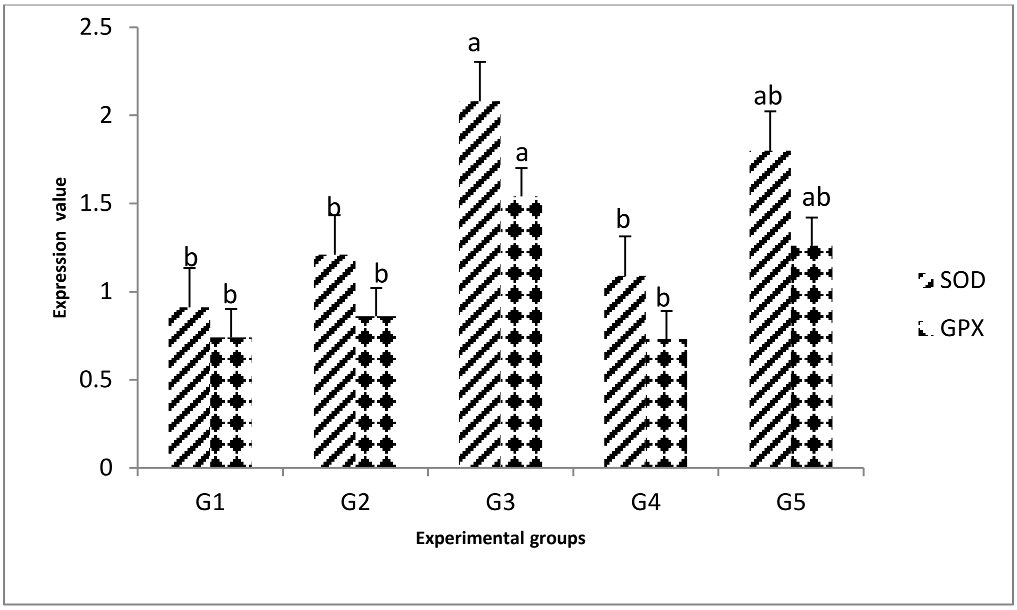

Fig. 3. Relative expression of antioxidant markers in sunflower and roselle seed meals supplemented groups. G1= control group; G2= sunflower meal 5%; G3= sunflower meal 10%; G4= roselle seed meal 5%; and G5= roselle seed meal 10%. Small alphabetical letters show significance when (P<0.05).

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تقييم مدي استخدام مستخلص عباد الشمس ومستخلص بذور الكركديه كمصادر بروتين غير تقليدية في النظام الغذائي لطائر السمان الياباني على أداء النمو، وخصائص الذبيحة، ومستقلبات المصل، والتعبير الجيني، والكفاءة الاقتصادية

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هدفت هذه الدراسة إلى تقييم تأثير استبدال كسبة فول الصويا في النظام الغذائي لطيور السمان اليابانية النامية بمستخلص عباد الشمس و بمستخلص بذور الكركديه عند مستويات (5 و 10%) كمصادر بروتين غير تقليدية. غطى التحليل أداء النمو، وصفات الذبيحة، وبعض مستقلبات مصل الدم، والتعبير الجيني، ومقاييس الكفاءة الاقتصادية. عند مقارنة طيور السمان في المجموعات التجريبية بتلك الموجودة في المجموعة الضابطة، لم يكن هناك فرق واضح من حيث وزن الجسم النهائي أو زيادة وزن الجسم أو نسبة التحويل الغذائي . تشير الفروق غير المعنوية إلى أن مصادر البروتين البديلة هذه كانت على الأقل بنفس فعالية كسبة فول الصويا في دعم أداء طائر السمان في الظروف المدروسة.

ظهرت قياسات مصل الدم عدم وجود فروق ذات دلالة إحصائية في مستويات البروتين الكلي، HDL، وبيروكسيد الدهون بين المجموعتين التجريبية والمجموعة الضابطة. ومع ذلك، فمن المهم أن جميع المجموعات التجريبية لديها مستويات أعلى من الكولسترول من المجموعة الضابطة. تم تنظيم الشكل التعبيرى بالجينات GH وIGF-I وPGAM2 وIFN-α وIL-2 وIL-4 وIL-6 وIL8 وSOD وGPX بشكل كبير في مجموعات SFM أو RSM بنسبة 10% مقارنة بالمجموعات الأخرى. لكن جين MSTN أنتجت الاتجاه المعاكس. وقد تم دراسه الكفاءة الاقتصادية الهامة حيث ظهرت أفضل النتائج مع اتباع نظام غذائي يتضمن 10% من مستخلص عباد الشمس يليه10% من مستخلص بذور الكركديه. في الختام، أدى استخدام مستخلص عباد الشمس ومستخلص بذور الكركديه ، وخاصة عند مستوى 10٪ بدلا من فول الصويا، إلى تعزيز أداء النمو، وتحسين التعبير عن العلامات المرتبطة بالنمو والمناعة ومضادات الأكسدة في السمان الياباني. وبالإضافة إلى ذلك، أدى ذلك إلى تحسين الكفاءة الاقتصادية.