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Original Article

The Effect of Moringa Oleifera Leaf and Canola Seed Powder on Fattening, Laying Performance, Blood Plasma Constituents, and Microbiota in Japanese Quails

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ABSTRACT

The aim of the study was to investigate the effect of feeding Japanese quail chicks with diets containing different levels of *Moringa oleifera* leaf and canola seed powder on growth performance, carcass yield, blood plasma constituents, and egg production. The trial lasted for a total of 7 weeks, with 5 weeks of fattening and two weeks of laying. The first group was the control group, while the second group was supplemented with *Moringa oleifera* leaves (4g/kg diet), the third group was supplemented with canola seed powder (4g/kg diet), the fourth group was supplemented with a mix of *Moringa oleifera* leaves and canola seed powder (8g/kg diet). The results showed that canola seed powder from 0-3 weeks of age increased body weight in comparison to the control group, but there were no significant differences (p<0.05) among groups in terms of the final body weight and feed conversion ratio. Average daily feed intake was significantly different (p<0.05). However, body weight, carcass weight, liver weight, gizzard weight, and abdominal fat weight increased significantly compared to the control group. Feeding *Moringa oleifera* leaves and canola seed powder significantly increased the total plasma protein, as compared to the control group. There were significant decreases in cholesterol, triglycerides and HDL levels among groups, with no significant differences in glucose, ALT and LDL among all treatments. The addition of *Moringa oleifera* leaf and canola seed powder showed significant effects on calcium and magnesium. Both *Moringa oleifera* leaf and *Moringa*-canola mixture significantly reduced the presence of some pathogenic bacteria in the digestive system, which was seen as an important contribution to the digestive and immune systems.

INTRODUCTION

Japanese quail are study birds that grow in small cages and are inexpensive to keep. They are used as a laboratory animal for research in biomedical sciences and as a pilot animal for poultry production because of its small body size, high egg production, and short generation interval (Padgett & Ivey, 1959 and Wilson *et al.*, 1961). Japanese quails mature in about 6 weeks, and are usually in full egg production by 7 weeks of age. Meat and egg production are a significant and major component of animal production in Turkey, due to its high economic contribution and its adaptability to new sectoral developments and consumer demands (Demircan *et al.*, 2010). In order to overcome some of the obstacles in the way of their development, it is necessary to not only find cheap sources of protein or energy, but also to search for feed additives with high medicinal value or quality in terms of protein, vitamins, and energy. Herbs and their metabolites (known as bioactive substances) play an important role as feed supplement. These

bioactive compounds help enhance animal health and productivity, supporting the production of safe and healthy products (Aldine, 2014).

Moringa oleifera, also called the "tree of life" or "miracle tree", is a tree native to India and abundant in Northern Iraq. *Moringa oleifera Lam.*, which belongs to the family *Moringaceae* was chosen for this study due to its fast growth, high nutritional value, use as a forage crop for livestock. (Nouman *et al.*, 2013), and for its multipurpose health effects (Worku, 2016). *Moringa oleifera* leaves are widely used traditionally for their antimicrobial (Suarez *et al.*, 2005) and pharmacological properties (Mehta *et al.*, 2003), and they appear to reduce the activity of pathogenic bacteria and molds and improve the digestibility of other foods, thus helping chickens to express their natural genetic potential (Gaia, 2005). 2012). *Moringa oleifera Lam* contains 30.3% crude proteins, including 19 amino acids (Moyo *et al.*, 2011), 2273–2978 kcal/kg dry matter metabolizable energy (Olugbemi *et al.*, 2010), and 0.6–11.2% minerals and vitamins (A, B, C, and E) (Mbora *et al.*, 2004). A series of researches have been conducted to study the effect of this leaf meal on the growth performance of layer and broiler chicks (Melesse *et al.* 2011), and on growth performance, carcass indicators and blood indices (Nuhu, 2010).

Canola is a winter crop originally derived from different types of rapeseed varieties that has been altered by genetic selection, markedly reducing its detrimental components (erucic acid and glucosinolate) to the negligible level of less than 20 µg g-1 (Harker *et al.*, 2015). Despite their high indigestible carbohydrate contents, canola meal is a suitable protein source for monogastric animal diets. Canola seeds are an excellent source of monounsaturated acids and linolenic acid is actually canola oil; the increase it is known to cause in the omega-3 levels of broilers' tissues and eggs tissues are beneficial for human health (An, Guo, Ma, Yuan, & Liu, 2010).

In poultry, the main sites of bacterial activity are the crop, the caeca and, to a lesser extent, the small intestine. Digestive system microorganisms can have a positive effect by releasing nutrients that the host can absorb in the gut and cecum, with the latter also transporting carbohydrates and amino acids (Moreto & Planas, 1989). (Xia *et al.* 2004) stated that the total viable *Escherichia coli* and *Clostridium* numbers in the intestine and caeca of chickens showed significant changes depending on the ration structure and copper contents. Beneficial bacteria can compete with pathogens to protect birds against them, and the flora plays a positive role in the development of the intestinal immune system. Moreover, the pH in the small intestine and caecum is an important indicator of the digestion of nutrients and the control of some bacteria.

The objective of this study is to study the effect of *Moringa oleifera* leaf and canola seeds on performance traits (live body weight, body weight gain, feed intake, feed conversion ratio), egg production, carcass yields (liver, gizzard, heart, abdominal fat, cloacal burse), and blood parameters (Total protein, Total cholesterol, Triglycerides, Glucose, ALT, HDL, LDL, Calcium, Phosphorus, Magnesium) on Japanese quails. Additionally, this study also investigated the effects of these additives on beneficial and useless bacterial flora and pH in the small intestine and caecum.

MATERIAL AND METHODS

The procedure of this trial was approved by the Animal Ethic Committee of the College of Agricultural Engineering Sciences, Harran University (Decision dated 11.11.2021 and numbered 79545). The experiments were carried out between October 17 and December 06, 2021, at the quail farm of Harran University, Faculty of Veterinary Medicine in Şanlıurfa Province, which is located at 37° 9′ 32.9364 latitude and 38° 47′ 48.8724 longitude.

Sample Collection and Preparation

The *Moringa oleifera* leaf meal used for this study was collected from the Department of Plant Production farm, Agricultural Research Centre, Slemani, Iraq. The harvested leaves were air dried in shade under a shed until they were crispy to touch, while retaining their greenish coloration. The dried leaves were then milled to produce *Moringa oleifera* leaf meal suitable for incorporation into chicks' diets. Canola seed was collected from Izmir in Turkey, and powdered with a grinding machine until it was suitable for incorporation into chicks' diets.

Experimental birds and design

In total, 240 day-old Japanese quails were divided into 4 main groups, with each group being divided into 3 replications. Each replication comprised 20 quails. The experiment lasted for 7 weeks and they were kept in cages (90cm × 40cm × 40cm) throughout this time. The temperature was controlled using a Thermostat; it was set between 34-36 °C in the first weeks, but was lowered by 2 °C per week after the second week.

It was then kept constant throughout the rest of the experiment (22 °C). Illumination was set to 23 hours of light, 1 hour of darkness/day. Birds in the control group were given the basal diet, the second group was given 4g of *Moringa* leaf powder per kilogram of basal ration, the third group was given 4g of canola seed powder per kilogram of basal ration, and the quails in the fourth group were given 4g of *Moringa* leaf powder + 4g of canola seed powder per kilogram of basal diet. The basal diet consisted of a three-stage feeding schedule: starter food from 1-14 days, grower food from 15-28 days, and finisher food from 29-53 days. Feed and water were provided throughout the trial period.

Fattening Performance

Every week live body weight, feed intake, and feed conversation ratio were measured. Daily egg production was recorded for 14 days.

At the end of the experiment period, 6 chicks from each replicate (3 females and 3 males) were randomly selected for each dietary treatment. They were fasted for 6 hours, weighed to determine live body weight, and then slaughtered by a sharp knife for complete bleeding, and immersed in boiling water for defeathering. Feathers were plucked, and head, shanks, and viscera were removed. Carcass, heart, gizzard, liver, abdominal fat, and cloacal bursa were weighed.

Blood Plasma Parameters

At the end of the growth period, blood samples were collected from 18 birds per treatment (6 birds/ replicate group). These blood samples of 1 ml/bird were stored in EDTA tubes and centrifuged (3,000 rpm – 10 min). After the plasma was separated, it was stored at -20 °C until the analysis. Total proteins, cholesterol, triglycerides, glucose, alanine aminotransferase, high density lipoprotein, low density lipoprotein, calcium, phosphorus, and magnesium were measured using standard protocols of commercial laboratory kits.

pH measurement and Microbiological analysis

The pH was determined immediately by collecting the small intestine and cecum contents of the quails and using a pH meter. The caeca of slaughtered animals were taken for microbiological analysis. 10 g of contents were taken from each caecal specimen with the help of a sterile spatula under aseptic conditions and left in sterile sampling bags (Bag Filter, France). Then, 90 ml of 0.1% sterile peptone water (LABM) was added to these bags and homogenized for 1 minute in a stomacher device (Easy Mix, France). In order to determine the number of microorganisms in each sample, dilutions of 10:1, 10:2, 10:3, 10:4, 10:5, 10:6, 10:7, 10:8 and 10:9 were made in these tubes.

Statistical analysis

Since there was no interaction between sex and groups, performance and digestive flora data were analyzed together. The influence of dietary treatments was examined using analysis of variance, while differences among means were evaluated using Duncan's multiple range test at 5% probability with the aid of software SPSS (2006). However, statistical analyzes were made according to the factorial trial design, since the group and sex were considered interactively in other analyzes.

RESULTS AND DISCUSSION

Growth Performance

Body weight results: The results are presented in (Table 1). There was no significant difference ($p<0.05$) in body weight between experimental groups and the control. However, the results indicated that weight gain was not significant ($p<0.05$) among all treatments. The results were in agreement with the findings of other researchers (Zeb *et al.*, 1999; Summers *et al.*,1988). This is most likely due to the presence of fish meal, which maintains amino acid moderation and prevents exchanges in anion-cation balancing at the ration (Banjo 2012). The results were in agreement with Paguia *et al.* 2014, who found that using 0.20% ,0.30%,0.40%, and 0.50% of *Moringa Oleifera* leaves in broiler diets did not significantly ($p<0.05$) influence broilers' body weight and body weight gain. These results were not in agreement with the findings of (Banjo, 2012), who reported that the inclusion of *Moringa oleifera* leaf meal at 1.2 and 3% levels in the diet of the broilers significantly ($p<0.05$) enhanced their weight gain in 1%, which was significantly higher than the control. Furthermore, the body weight gain of chicks increased with the increasing percentages of both *Moringa* leaf and canola seed powder during the finisher and total periods. (Teteh *et al.*, 2013) showed that overall chick weights and daily body weight gain increased significantly ($p<0.05$) with age when 1 and 2 % *Moringa oleifera* leaves were used on broilers as compared to the control group. (Makanjuola *et al.*, 2014) indicated that adding *Moringa oleifera* leaves to

broiler diets at 0.2, 0.4 and 0.6% for 28 days had no adverse effect on the final weight and body weight gain in broiler chickens.

The effect of feeding different levels of *Moringa oleifera* leaf meal and canola seed powder are shown in Table 1. The average feed intake during ages of 1-7 weeks were significantly higher in the 0.4% *Moringa oleifera* leaf, 0.4% canola seed powder and 0.4% (*Moringa oleifera* leaf meal + canola seed powder) groups as compared to the control treatment. Supplementation of the basal diet with *Moringa oleifera* leaves at different levels (1 and 3%) resulted in an improve of Feed Conversion Ratio (Abou-Elezz *et al.*, 2011). This may be attributed to birds fed *Moringa Oleifera* based diets adequately utilizing the nutrients they consumed. The results coincided with the finding of (Ebenebe *et al.*, 2012), who reported that chicks fed *Moringa oleifera*-based diets performed significantly ($p<0.05$) better than birds of the control group in terms of weight gain, feed intake, and feed conversion ratio.

Table 1 – Effects of *Moringa oleifera* leaves and canola seed powder on daily live weight gain, daily feed intake, feed conversion ratio, and egg production of Japanese quails.

	Weeks	Control	*Moringa*	Canola seed	*Moringa*+Canola	SEM	*p*-Value
Daily live weight gain, g day^{-1}	0-3	3.55^{a}	3.67^{ab}	3.78^{b}	3.65^{ab}	0.07	0.15
	4-7	2.42^{a}	2.44^{a}	2.64^{a}	2.85^{a}	0.23	0.46
	0-7	4.04^{a}	4.10^{a}	4.17^{a}	4.25^{a}	0.08	0.28
Daily feed intake, g day-1	0-3	10.54^{a}	11.76^{c}	11.31^{b}	11.51^{bc}	0.09	0.01
	4-7	23.76^{a}	24.67^{b}	25.54^{c}	24.78^{b}	0.11	0.01
	0-7	18.09^{a}	19.14^{b}	19.45^{c}	19.09^{b}	0.09	0.01
Feed conversion ratio (g feed/ g weight)	0-3	2.99^{a}	3.24^{c}	3.03^{ab}	3.21^{ab}	0.06	0.01
	4-7	12.35^{a}	12.16^{a}	10.01^{a}	11.74^{a}	1.73	0.76
	0-7	4.56^{a}	4.74^{a}	4.74^{a}	4.55^{a}	0.09	0.22
Egg production	0-14	55.66^{a}	64.16^{a}	54.30^{a}	62.12^{a}	4.80	0.54

These values were calculated considering 7 females. Since there was no interaction between sex and groups, performance data were analyzed together. SEM: Standard error mean. abc: Differences between averages in the same column with different superscripts are significant.

Carcass characteristics

Statistical analyses of the carcass yield of different groups are shown in Table 2. The dietary supplementation of *Moringa Oleifera* and canola seed powder caused significant differences ($p>0.05$) between experimental and control groups for live weight, carcass, liver, gizzard, and abdominal fat. The increment in abdominal fat weight with the increased supplementation level of *Moringa Oleifera* might be due to the higher level of fat content in *Moringa* seeds as observed by (Compaoré *et al.*, 2011). Moyo *et al.*, 2012 reported similar results showing significantly higher values when using a *Moringa Oleifera* and canola seed powder treatment, while the present results are not in agreement with Elwinger (1986). On the other hand, heart and cloacal bursa did not present significant differences ($p>0.05$) when using *Moringa Oleifera* and canola seed powder as compared to control group. These results may be due to the capacity of *Moringa Oleifera* to regenerate damaged hepatocytes and pancreatic cells via its antioxidant properties (Abd El Latif *et al.*, 2014). In spite of these results, the numerical increase in liver weight has probably been due to the lower fat content of the diet, which makes the liver subject to producing energy from carbohydrates and causes its weight to be higher in comparison with the control group. Fat can supply a major part of required energy and will decrease liver demand for lipogenesis (Leeson & Summers, 1997).

Table 2 – Effects of supplementation of *Moringa oleifera* leaves and canola seed powder on the carcass yield of Japanese quails.

	Control	*Moringa*	Canola	*Moringa*+canola	SEM	*p*-Value
Live weight, g	200.00	210.00	221.16	218.88	5.96	0.06
Carcass, g	136.16	139.55	146.28	150.31	4.09	0.08
Gizzard, g	3.14	3.32	3.42	3.59	0.12	0.08
Liver, g	3.91	3.96	4.99	4.61	0.38	0.14
Heart, g	1.83	1.90	1.90	1.88	0.07	0.89
Abdominal fat, g	2.12	3.79	2.46	4.11	0.55	0.06
Cloacal Bursa, g	0.22	0.64	0.16	0.19	0.14	0.49

SEM: Standard error mean; P: The level of importance. Multiple comparisons were not made in factorial trial designs.

Egg production

The average total eggs laid for 14 days was highest when using *Moringa* leaf, while the least was reported when using canola seed powder. There was no significant difference in egg production between the various levels (p<0.05). Similarly, previous results in Japanese quails have shown that *Moringa oleifera* and canola seed powder had no significant differences (p<0.05) for egg laying (Abou-Elezz *et al.*, 2012). However, (Olugbemi *et al.*, 2010) observed that supplementing 5% and 10% dried *Moringa* leaves in laying hen diets had no effect on egg production, while (Abdel-Wareth & Lohakare 2021) indicated that supplementation of *Moringa oleifera* leaves (3, 6 and 9 g/kg) in Hy-Line Brown hens diet significantly increased egg weight, production, and mass between 64–68, 68–72, and 64–72 weeks of age.

Plasma biochemical assay

Data of plasma biochemical indices for Japanese quails are presented in Table 3. The results showed that supplementation of *Moringa oleifera* and canola seed powder significantly (p<0.05) increased total plasma protein, the highest values of which were in the *Moringa oleifera* and *Moringa*+ canola seed groups. This result may be due to the enhanced humoral immune response induced by the several rich phytoconstituents in *Moringa oleifera* and canola seed powder (Aja *et al.*, 2014), the abundance of calcium and vitamin A present in *Moringa oleifera* (Leone *et al.*, 2015), as shown in hen egg albumen (Wei Lu *et al.*, 2016), or still to the decrease of protein glycation and protein oxidation caused by the polyphenols present in *Moringa oleifera* (Al-Malki & El Rabey 2015). Furthermore, both triglycerides and cholesterol decreased with the dietary treatments *Moringa oleifera* and canola seed powder compared with the control group. These results may be due to reduced oxidative stress and greater glycation that maintains lipid homeostasis (Sangkitikomol *et al.*, 2014), or to less inflammation and lower hepatic stellate cells involved in the progression of liver fibrosis (Leone *et al.*, 2016).

ALT and glucose were not affected by either *Moringa oleifera* or canola seed powder compared with the control group. (Yuangsoi *et al.*, 2014) found that the levels of ALT and AST were similar in all diets, indicating normal organ function upon feeding birds with *Moringa oleifera*. HDL values were higher in females except in the canola seed group, while LDL and Ca values were lower in females, except for the *Moringa*+canola mix groups. Females receiving *Moringa* and Canola had a positive HDL response, while LDL values decreased (Table 3). Additionally, lower LDL cholesterol values

Table 3 – Effects of supplementation of *Moringa oleifera* leaves and canola seeds powder on plasma biochemical assay of Japanese quails.

Control		*Moringa*		Canola		*Moringa*+Canola			*p*-Value		
Female	Male	Female	Male	Female	Male	Female	Male	SEM	Groups	Sex	Group× Sex
Glu (mg/dl)											
214.00	205.11	194.40	218.56	211.00	244.13	238.86	198.17	21.20	0.77	0.89	0.30
ALT (U/L)											
7.08	7.02	7.16	6.95	7.12	7.47	7.45	6.96	0.52	0.96	0.78	0.88
TP (g/dL)											
2.15a*	2.18a	3.27c	5.01c	2.50a	2.43a	3.27b	3.93b	0.40	0.01	0.04	0.10
TAG (mg/dl)											
328.60	72.89	481.60	77.11	377.00	91.75	181.29	381.83	79.49	0.69	0.02	0.02
TC (mg/dl)											
88.80	1 43.89	91.60	152.00	88.75	146.13	171.57	135.33	19.74	0.19	0.01	0.04
HDL (mg/dl)											
53.40	97.56	45.80	109.00	42.25	100.13	125.57	83.67	15.17	0.12	0.01	0.01
LDL (mg/dl)											
42.16	31.76	50.88	27.58	72.55	27.65	15.37	38.37	9.99	0.17	0.06	0.01
Ca (mg/dl)											
10.16	6.24	11.22	6.66	11.73	7.00	7.06	8.20	0.86	0.21	0.01	0.01
P (mg/dl)											
4.96	4.69	6.08	4.38	4.98	4.50	4.50	3.68	0.65	0.36	0.08	0.69
Ma (mg/dl)											
1.98	1.44	2.52	1.52	2.10	1.71	1.74	1.70	0.21	0.37	0.01	0.15

The letters shown with superscript indicate the differences according to the groups. Multiple comparisons of sex and group×sex interaction could not be made. SEM: Standard error mean; P: The level of importance. Multiple comparisons were not made in factorial trial designs. ALT; alanine aminotransferase, HDL; high density lipoprotein, LDL; low density lipoprotein.

were observed in quails receiving *Moringa oleifera* and canola seed powder compared to the control group, but this was not significant (p>0.05). The main function of insoluble fiber is to bind bile acids, which reduces the absorption of fat and cholesterol. (Joshi & Mehta, 2010). Moreover, there were significant differences (p<0.05) in HDL between sex in the same group and sex in different groups. HDL helps to rid the body of excess cholesterol so it is less likely to end up in arteries, making cholesterol metabolism more accelerated and decreasing its circulating levels (Francis *et al.*, 2002). However, supplementation of *Moringa oleifera* and canola seed powder had significant (p<0.05) effects on calcium and magnesium levels, but was not significant for phosphorus. *Moringa oleifera* and canola seed have been proven to be good sources of fat, proteins, antioxidants and minerals, hence they could be useful to treat malnutrition due to micronutrients deficiency in children (Compaoré *et al.*, 2011).

Intestinal pH and Flora

The pH values and some bacterial count values in the small intestine and cecum of the experimental groups are given in Table 4. According to the results of the study, it was seen that the pH of the small intestine and caecum contents changed according to the ration type. Many studies have concluded that the cecum was more alkaline than the small intestine (Shakouri *et al.*, 2008). Similar results emerged in our study, and it was shown that the nature of the feeding had a significant effect on the pH of the digestive system. The pH of the digesta from the small intestine and cecum differed according to the type of additive (p<0.001; Table 4). *Moringa* and canola decreased the pH only in the small intestine, but birds receiving the *Moringa* + Canola mixture based diet had a lower pH than those fed the control, *Moringa*, and canola-based diets.

In the study, it was observed that the *Moringa* and canola mixture caused a significant decrease (p<0.001) in the formation of *E. coli*, yeast, and mold. This may be attributed to the lower pH in the caeca of quails on these diets. (Gabriel *et al.*, 2006) stated that the microbiota may have a beneficial effect on nitrogen metabolism, and is also significantly dependent on dietary protein. (Muramatsu *et al.*, 1987) stated that protein needs are higher for conventional chickens than for germ-free chickens. The leaves of the *Moringa* plant have a high protein content (26.4%), while the canola plant has both high protein (23%) and high oil content. The effect of both the *Moringa* plant and the *Moringa*+canola mixture on reducing the total number of bacteria may be related to the reduction of some pathogenic bacteria and molds. On the other hand, it is possible that the high oil content in Canola seeds (45%) suppresses some microorganisms. We speculated that there was no reduction in the beneficial bacteria population here. This change in the bacterial population may also have affected immune systems, since the intestinal region is an important part of the immune system and there were no cases of illness or death during the experiment, especially in the *Moringa* and *Moringa*+canola groups.

CONCLUSION

It was concluded that *Moringa oleifera* leaves and canola seed powder improved feed intake, carcass yield, and total plasma protein, and decreased total cholesterol and triglycerides. Traditional farmers must be encouraged to grow fast, easy growing *Moringa* trees and use leaves in poultry feed because of their rich nutrients and bioactive phytochemical compounds. Moreover, at the end of the study, it was determined that the *Moringa* plant caused a significant decrease in

Table 4 – The composition of some microbiota determined by pH and microbiota count in the contents obtained from the small intestine and caecum of quails' digestive systems.

Groups	Control	*Moringa*	Canola seed	*Moringa*+Canola	SEM	*p*-Value
S PH	6.36a	6.47b	6.51c	6.35a	0.02	0.001
C PH	7.04c	7.05c	6.59b	6.45a	0.06	0.0001
Lactic acid	19.23a	73.71c	52.8b	-	9.12	0.001
Acetic acid	9.55d	33.8a	13.6b	-	2.90	0.001
LAB	8.04a	10.5c	10.6d	9.12b	0.24	0.0001
Total bacteria	10.21c	10.01a	10.92d	10.18b	0.07	0.0001
Entrobacter	8.59a	8.49b	7.71d	8.86c	0.09	0.001
Col. Bacteria	8.56b	8.32a	7.68d	8.77c	0.08	0.001
E. coli	7.94c	7.91c	7.34b	3.37a	0.39	0.0001
Yeast	4.47c	6.69d	3.71b	3.61a	0.26	0.001
Mold	2.90c	3.08d	2.37b	2.08a	0.08	0.001

Lactic acid and acetic acid, g kg-1; S pH: small intestine pH; C PH: caecum pH; LAB: Lactic Acid Bacteria; Col bact.: coliform bacteria; CFU g^{-1}: the number of colony-forming units per gram. abcd: Differences between averages in the same column with different superscripts are significant.

total bacteria, and the mixture of *Moringa* and canola provided a significant decrease in the formation of *E. coli*, yeast, and mold in the total digestive system. It can be said that the mixture of *Moringa* plant +canola and *Moringa* is very effective in suppressing some pathogenic bacteria.

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AUTHORS' CONTRIBUTIONS

BJ: data collection, project design and drafting the article; SY: supervisor of the project and statistical analysis.

CONFLICT OF INTEREST DECLARATION

There is no conflict of interest.

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