



## Effect of *Trichilia monadelpha* Stem Bark Extract on the Fatty Acid Composition of Rabbit's Thigh Meat

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| Article information  | Abstract  |
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| <b>History</b><br><br>Received 08/10/2022<br>Accepted 18/10/2022<br>Published 23/10/2022   | <i>This study was conducted to evaluate the effects of Trichilia monadelpha stem bark extract on the fatty acid composition of rabbit's thigh meat. A total of 40, 6-7 weeks New Zealand white × Chinchilla crossbred rabbits with an average weight of 490 ± 4.40 were randomly divided to 4 groups with 5 replications consisting of 2 animals each in a completely randomized design. Rabbits in treatment 1 (T1) was fed basal diet with no Trichilia monadelpha stem bark extract (TMSB), T2, T3 and T4 were fed basal diet with 3mL, 6mL and 9mL TMSB per rabbit/day. Phytochemical analysis of TMSB revealed the presence of tannins (10.95 mg/g), alkaloids (9.22 mg/g), saponins (4.75 mg/g), oxalates (3.10 mg/g), flavonoids (15.88 mg/g), phenols (18.46 mg/g), terpenoids (8.62 mg/g), glycosides (7.11 mg/g) and 2-diphenyl 1-picrlyhydrazide (430.8 mg/g). Concentrations of vitamins identified in the extract are; vitamin A (2.190 mg/100g), vitamin B1 (0.567 mg/100g), vitamin B2 (0.301 mg/100g), vitamin B3 (0.227 mg/100g), vitamin B9 (0.080 mg/100g), vitamin B12 (0.209 mg/100g) and vitamin C (5.680 mg/100g). Composition of saturated fatty acid (TSFA) decrease with a significant increase in monosaturated fatty acid (MUFA) and polyunsaturated fatty acid (PUFA) (P&lt;0.05) across the treatments. It was found that TMSB significantly (P&lt;0.05) influenced the concentration of total unsaturated fatty acid (TSFA). Therefore, it can be concluded that TMSB is capable of modulating the fatty acid and improving the quality of meat from rabbit without causing any deleterious effect on the performance of the animal.</i> |
| <b>Keywords</b><br><br><i>Trichilia monadelpha, fatty acid, phytochemicals, vitamins, meat, food safety</i>  |   |
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### 1. Introduction

The use of plant extracts or phytochemicals have been gaining increasing attention in the feed industry due to their beneficial effect on livestock performance and food safety (IDT, 2000). Phytochemical feed additives (PFAD) comprise of a wide range of plants like herbs, spices and plant derived essential oils with marked pharmacological functions (IPP, 2011; Agubosi *et al.*, 2021). The chemical composition of PFAD underlies a certain variation due to their bioactive compounds and other factors like anti-nutrients, species, location, harvesting and storage conditions (Agubosi *et al.*, 2022). Extracts from plants contain secondary metabolites or phytochemicals which performs several therapeutic activities such as: antimicrobial against bacteria (gram-positive and gram-negative), antioxidants (scavenging free radicals), immune-modulatory, hypolipidemic, cytotoxic antiviral, anti-plasmodial, anti-trypanosomal and hepato-protective (Mota *et al.*, 1985; Kamanzi *et al.*, 2004). The medicinal use of *Trichilia monadelpha* can never be overemphasized as phytochemical screening revealed the presence of terpenoids, saponins, alkaloids, flavonoids, tannins, oxalates and glycosides (Buzzini *et al.*, 2008; Savithramma *et al.*, 2012). The plant belongs to the family Meliaceae with over 90 species and widely distributed in several parts of Asia and Africa (Savithramma *et al.*, 2012). Aqueous extract of the leaves, stem bark and root are used in traditional medicine for the treatment of gastro-intestinal disease, sexually

transmitted disease, malaria, pneumonia and other respiratory disease (Woode *et al.*, 2012). Many studies have shown the antibacterial, antifungal, anti-proliferative, antiviral, anti-helminthic, cytotoxic, antioxidant, anti-inflammatory, antitumor, antioxidant, anti-depressant, analgesics and antipyretic properties of both aqueous, methanolic and ethanolic stem bark extract (Ivo *et al.*, 2014; Aladesanmi and Odediran, 2000). The antimicrobial effect of *Trichilia monadelpha* stem bark extract against *Escherichia coli*, *Staphylococcus aureus*, *Staphylococcus pyogenes*, *Bacillus aereus*, *Pseudomonas spp*, *Aeromonas spp* and *Klebsiella spp* was reported by Germano *et al.* (2007). Recent results from Rababah *et al.* (2006) confirm that animals fed phytogenic feed additives showed higher meat PUFA content. This effect was also correlated with a reduction in the susceptibility of the chicken meat to lipid peroxidation. Sanchez *et al.* (2012) also reported a higher concentration of omega-3 and omega -6 fatty acid in the thigh meat of rabbits fed *Hypericum perforatum* L. extract. For this reason, animal nutrition is currently evolving toward PUFA-enriched diets to improve animal fat healthfulness (food safety) (Bourre, 2005). Therefore, feeding animals with *Trichilia monadelpha* stem bark extract will provide potential benefits to both the animal and consumers due to its abundant potentials.

## 2. Materials and Methods

### 2.1 Experimental site and collection of plant material

The experiment was carried out at Sumitra Research Institute India located within with the coastline of 1,600 Km, 23° 13'N 72°41'E in the month of March to July, 2022. *Trichilia monadelpha* stem bark was collected within the premises of Sumitra Research Institute, India and the taxonomic identification was carried out by a certified taxonomist at the Department of Crop Science of the same institute where a voucher specimen (ST/0221H) was submitted.

### 2.2 Preparation of *Trichilia monadelpha* stem bark and analysis

*Trichilia monadelpha* stem bark was cut into smaller pieces, washed with running clean water and shade dried for 10 days in an aluminum pan, grinded into powder with an electric blender and transferred into an air tight labelled zip lock bag. 500 g of dried *Trichilia monadelpha* stem bark was extracted with 1000 mL of methanol in a conical flask for 48 hours at a temperature of 25°C and filtered with Whatman No.1 filter paper. Methanol was dried in a rotary evaporator under low pressure at a temperature of 40 °C and the extract (TMSB) was stored in the refrigerator at 4 °C. Sample of *Trichilia monadelpha* stem bark extract (TMSB) was taken to the laboratory and were analyzed for tannins (Ahmad *et al.*, 2014), phenols (Bhat *et al.*, 2018), terpenoids (Debnath and Vyas, 2015), saponins (Tiwari *et al.*, 2011), alkaloids (Jamuna and Paulsamy, 2013), oxalates and glycosides (Sczkowski *et al.*, 1998), Terpenoids and flavonoids (Harbone, 1973), vitamin C (Okwu and Ndu, 2006), vitamin A (Okwu, 2004), vitamin B complex (Robert *et al.*, 2003) and DPPH (diphenylpicrylhydrazyl) radical scavenging activity (Rao *et al.*, 2010).

### 2.3 Animal management and experimental design

A Completely randomized design (CRD) was used in this study. A total of 40, 6-7 weeks New Zealand white × Chinchilla crossbred rabbits with an average weight of  $490 \pm 3.7$  were sourced from a reputable farm in India. The animals were properly inspected against any deformity and transported early in the morning to Sumitra Research Farms. Rabbits were equally divided into 4 dietary treatments with 5 replications consisting of 2 animals each. Before the commencement of the experiment, hutches, watering and feeding troughs, were thoroughly cleaned, disinfected, and sprayed against parasites. Animals are placed on a 14 days adjustment period after balancing the weight and given prophylactic treatment of Oxytrox® L.A (antibiotics) and Ivermectin Injection (endo- and ecto- parasites) adhering strictly to the manufacturers recommendation. Other sanitary and management practices were taken throughout the experimental period which lasted for 84 days. Basal diet was prepared to meet the nutrient requirement of growing rabbits (NRC, 1977). Fresh clean water was provided at all times. The amount of feed consumed per rabbit was determined as the difference between the feed offered and leftover. Body weight gain (BWG) change was calculated as the difference between the final and initial body weight. Feed conversion ratio was determined as total feed intake divided by BWG. The experimental set-up follows the pattern below:

Treatment 1: Basal diet with no *Trichilia monadelpha* stem bark extract (TMSB); Treatment 2: Basal diet plus 3 mL TMSB per rabbit/day; Treatment 3: Basal diet plus 6 mL TMSB per rabbit/day; Treatment 4: Basal diet plus 9 mL TMSB per rabbit/day

## 2.4 Laboratory analysis

Proximate analysis of the experimental diet was carried out using ProxiMate™ NIR instrument (India) with dimensions (WxDxH) (260 × 435 × 500 mm), 900 – 1700 nm wave length range, frequency (50/60 Hz), power consumption of 60W and resolution VIS (10 nm). Fatty acid analysis was carried out on 5 randomly selected rabbits using fatty acid analyzer (YL 6500 series) with the following specifications: column: TR-WAX (30m × 25mm × 0.25µm), carrier gas: helium, 1.0 ml/min (split ratio 50:1), injector: capillary 270°C, detector: FID 270°C, sample preconditioning: FAME and injector volume: 1.0 µl (liquid).

## 2.5 Statistical analysis

Data were subjected to a one-way analysis of variance (ANOVA) by using the general linear model procedure of SAS (Version 9.4, SAS Institute Inc., Cary, NC). When the effect was declared significant ( $p < 0.05$ ), treatment means were compared using a Duncan's multiple-range test.

**Table 1:** Gross composition of experimental basal diets

| Ingredient                     | Quantity (Kg) |
|--------------------------------|---------------|
| White corn                     | 35.60         |
| Wheat offal                    | 23.00         |
| Palm kernel meal               | 14.00         |
| Soy bean meal                  | 15.00         |
| Di-calcium phosphate           | 5.30          |
| Palm oil                       | 5.00          |
| Salt                           | 0.35          |
| ‡Premix                        | 0.25          |
| Toxin binder                   | 0.20          |
| Methionine                     | 0.10          |
| Lysine                         | 0.20          |
| Total                          | 100.0         |
| Calculated analysis (g/kg DM)  |               |
| Dry matter                     | 900.46        |
| Crude protein                  | 170.10        |
| Ash                            | 19.11         |
| Crude fibre                    | 150.40        |
| Ether extract                  | 10.88         |
| Metabolizable energy (kcal/kg) | 2890.22       |

\*Each 1 kg contains: 8,500 IU vitamin A, 3000 IU vitamin D<sub>3</sub>, 15.6 IU vitamin E, 2.10 mg vitamin K, 8.60 mg calcium pantothenate, 0.02 mg vitamin B<sub>12</sub>, 0.55 mg folic acid, 300 mg choline chloride, 30.02 mg chlorotetracycline, manganese 150.30 mg, 62.75 mg iron, 44.04mg zinc, 2.7 mg copper, 1.50 mg iodine, 0.34 mg cobalt, 0.11 mg selenium

## 3. Results And Discussion

### 3.1 Chemical composition of secondary metabolites in *Trichilia monadelpha* stem bark extract (TMSB)

Examination of the secondary metabolites (phytochemicals) in *Trichilia monadelpha* stem bark extract revealed the presence of eight bioactive compounds at different concentrations (Table 2). Phenols had the highest concentration (18.46 mg/g) followed by flavonoids (15.88 mg/g), tannins (10.97 mg/g), alkaloids (9.22 mg/g), terpenoids (8.62 mg/g), glycosides (7.11 mg/g), saponins (4.75 mg/g) and oxalates (3.10 mg/g) respectively. DPPH (diphenylpicrylhydrazyl) radical scavenging activity revealed that TMSB contained (430.8 mg/g). Phytochemicals are bioactive chemicals of plant origin used by plants for growth or defense against pathogens or predators (Saxena *et al.*, 2013; Alagbe *et al.*, 2020). They are also capable acting as co-factors and inhibitors of enzymatic reactions (Sarker and Nahar, 2007), substrate of biochemical reactions (Balandrin and Klocke, 1998; Alagbe *et al.*, 2017), scavengers of free radicals and selective inhibitors of deleterious pathogens (Hyun *et al.*, 2018; Cheeke, 2000). All the bioactive compounds recorded in TMSB have a marked pharmacological functions. For instance, phenol which is the most abundant compound contains one benzene ring with hydroxyl groups is effective in the reduction of reactive oxygen species (antioxidants) and promote a healthy gut in animals (Gadde *et al.*, 2017), flavonoids and terpenoids play major role in the selective growth of

beneficial bacteria (Lillehoj and Lee, 2013; Musa *et al.*, 2020), alkaloids function as antimalarial and analgesics (Akintayo and Alagbe, 2020), tannins possess antibacterial and antiviral activities (Daniyan *et al.*, 2010; Adewale *et al.*, 2021), saponins have been recommended to be required in antibacterial and anti-inflammatory activities (Abu *et al.*, 2016). High concentration of phytochemicals beyond permissible range can be deleterious to the body of animals. According to Chai and Liebman (2004), high oxalate diet can increase the risk of renal calcium absorption and has been implicated as a source of kidney stones. However, all values were within the tolerable level recorded for rabbits by Omokore and Alagbe (2019). DPPH (2, 2-diphenyl-1-picrylhydrazyl) test is used to ascertain the antioxidant properties in TMSB and ability to scavenge free radicals thus preventing diseases and infections. The DPPH in the test material is potent due to the presence of phytochemicals especially phenolic compounds (FAD, 2013).

**Table 2:** Chemical composition of secondary metabolites in *Trichilia monadelpha* stem bark extract (TMSB)

| Constituents | Composition (mg/g) |
|--------------|--------------------|
| Alkaloids    | 9.22               |
| Tannins      | 10.97              |
| Saponins     | 4.75               |
| Oxalates     | 3.10               |
| Flavonoids   | 15.88              |
| Phenols      | 18.46              |
| Terpenoids   | 8.62               |
| Glycosides   | 7.11               |
| DPPH         | 430.8              |

### 3.2 Vitamin properties of *Trichilia monadelpha* stem bark (TMSB)

Chemical analysis on the vitamin properties of *Trichilia monadelpha* stem bark (TMSB) revealed that it contained vitamin A (retinol) at 2.190 mg/100g, vitamin B1 (thiamin), vitamin B2 (riboflavin), vitamin B3 (niacin), vitamin B9 (cobalamin) and vitamin C (ascorbic acid) at 0.567 mg/100g, 0.301 mg/100g, 0.227 mg/100g, 0.080 mg/100g, 0.209 mg/100g and 5.680 mg/100g respectively. Vitamin C had the highest concentration while vitamin B9 had the lowest concentration. Vitamins are chemical compounds that are essential for body functioning, enzymatic activities and certain biological functions (Chawla and Kaur, 2004). Vitamin C is the most abundant compound in TMSB which is a clear indication that it can act as an antioxidant as well as the formation of iron needed for the driving of oxygen in all parts of the body (Mullis *et al.*, 2003). According to (Olkowski *et al.*, 1992), vitamin B is responsible for maintaining nerve function, production of red blood cell, synthesis of fats and carbohydrates as well as replication of deoxyribonucleic acid (DNA) while vitamin A helps in improving the immune system, muscle tissues and sharp vision (Campbell *et al.*, 2000; Engle *et al.*, 2001).

**Table 3:** Vitamin properties of *Trichilia monadelpha* stem bark (TMSB)

| Parameters                | Composition (mg/100g) |
|---------------------------|-----------------------|
| Vitamin A (Retinol)       | 2.190                 |
| Vitamin B1 (Thiamin)      | 0.567                 |
| Vitamin B2 (Riboflavin)   | 0.301                 |
| Vitamin B3 (Niacin)       | 0.227                 |
| Vitamin B9 (Folate)       | 0.080                 |
| Vitamin B12 (Cobalamin)   | 0.209                 |
| Vitamin C (Ascorbic acid) | 5.680                 |

### 3.3 Fatty acid composition of rabbit thigh meat fed different levels of *Trichilia monadelpha* stem bark extract

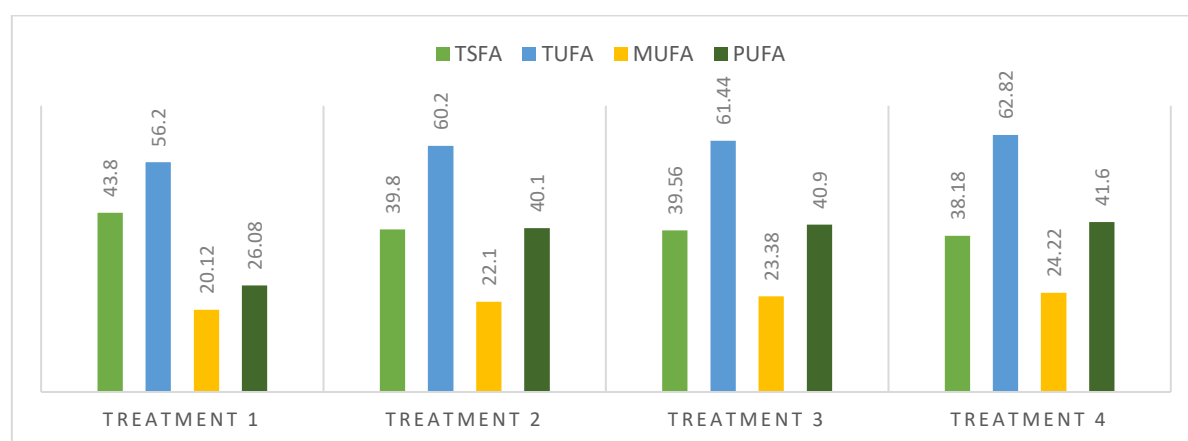
Fatty acid composition of rabbit thigh meat fed different levels of *Trichilia monadelpha* stem bark extract revealed the presence of total saturated fatty acid (TSFA), monosaturated fatty acid (MUFA) and polysaturated fatty acid (PUFA) which ranged from 38.18 – 43.80 %, 20.12 – 24.22 % and 21.60 – 41.60 % respectively (Table 4). TSFA, MUFA and PUFA values were significantly ( $P < 0.05$ ) influenced by the treatments. Meat samples from T2, T3 and T4 had the highest concentration of total unsaturated fatty acid (PUFA and MUFA) compared to the other treatment. Conversely, T1 had the highest concentration of TSFA compared to the other treatments ( $P < 0.05$ ). High PUFA and MUFA level in T2, T3 and T4 could be attributed to the presence of phytochemicals in TMSB which enhanced the absorption or stability of essential nutrients as well as increase in the shelf life of

products (Alagbe *et al.*, 2022; Davidson and Naidu, 2000). Increase intake of SFA results in cardiovascular disease in animals (Dawson *et al.*, 1995). The result obtained in this experiment is in agreement with the findings of Chouliara *et al.* (2007); Brannan (2008) but contrary to the reports of Anadon *et al.* (2002) this variation could be attributed to differences in the composition of phytochemicals of the test ingredients, method of processing or extraction as well as concentrations. Diet plays an important role in the overall health and performance of an animal. Furthermore, nutrition has a regulatory effect on biological processes in muscle, which can influence the quality of meat and meat products (Anderson *et al.*, 2005).

**Table 4:** Fatty acid composition of rabbit's thigh meat fed different levels of *Trichilia monadelph*a stem bark

| Constituents                        | T1                 | T2                 | T3                 | T4                 | SEM  |
|-------------------------------------|--------------------|--------------------|--------------------|--------------------|------|
| C14:0 (Lauric acid)                 | 4.06 <sup>a</sup>  | 2.17 <sup>b</sup>  | 2.11 <sup>c</sup>  | 2.00 <sup>c</sup>  | 0.06 |
| C16:0 (Palmitic acid)               | 19.4 <sup>a</sup>  | 11.3 <sup>b</sup>  | 10.7 <sup>c</sup>  | 10.3 <sup>c</sup>  | 0.42 |
| C18:0 (Stearic acid)                | 7.18 <sup>a</sup>  | 5.60 <sup>b</sup>  | 5.10 <sup>b</sup>  | 4.00 <sup>c</sup>  | 0.30 |
| C20:0 (Arachidic acid)              | 3.46 <sup>a</sup>  | 2.01 <sup>b</sup>  | 1.19 <sup>c</sup>  | 1.15 <sup>c</sup>  | 0.42 |
| C22:0 (Behenic acid)                | 0.55 <sup>a</sup>  | 0.38 <sup>b</sup>  | 0.18 <sup>c</sup>  | 0.10 <sup>c</sup>  | 0.02 |
| C14:1c (Myristic acid)              | 1.57 <sup>d</sup>  | 3.01 <sup>c</sup>  | 4.08 <sup>b</sup>  | 5.22 <sup>a</sup>  | 0.07 |
| C16:1c (Palmitoleic acid)           | 2.09 <sup>c</sup>  | 3.00 <sup>b</sup>  | 4.05 <sup>a</sup>  | 4.11 <sup>a</sup>  | 0.02 |
| C18:1c (Oleic acid)                 | 10.1 <sup>c</sup>  | 14.3 <sup>b</sup>  | 19.6 <sup>a</sup>  | 20.7 <sup>a</sup>  | 0.44 |
| C18:1n9t (Linoleic acid)            | 1.55 <sup>c</sup>  | 2.03 <sup>b</sup>  | 3.40 <sup>a</sup>  | 3.58 <sup>a</sup>  | 0.01 |
| C18:1n9c (Linolelaidic acid)        | 1.00 <sup>b</sup>  | 1.61 <sup>b</sup>  | 2.08 <sup>a</sup>  | 2.77 <sup>a</sup>  | 0.05 |
| C:22:1 (Erucic acid)                | 0.50 <sup>c</sup>  | 0.87 <sup>b</sup>  | 1.00 <sup>a</sup>  | 1.07 <sup>a</sup>  | 0.04 |
| C18:2n6 ( $\alpha$ -linolenic acid) | 18.7 <sup>b</sup>  | 21.9 <sup>a</sup>  | 24.6 <sup>a</sup>  | 25.9 <sup>a</sup>  | 0.11 |
| C20:5n3 (Arachidonic acid)          | 0.94 <sup>c</sup>  | 1.88 <sup>b</sup>  | 2.02 <sup>a</sup>  | 2.40 <sup>a</sup>  | 0.55 |
| C18:3n3 ( $\gamma$ -linoleic acid)  | 7.18 <sup>c</sup>  | 10.8 <sup>b</sup>  | 13.7 <sup>a</sup>  | 18.4 <sup>a</sup>  | 0.42 |
| C20:4n6 (Docosanoic acid)           | 2.05 <sup>b</sup>  | 2.86 <sup>b</sup>  | 4.05 <sup>a</sup>  | 5.71 <sup>a</sup>  | 0.08 |
| C20:3n6 (Eicosapentanoic acid)      | 1.34 <sup>b</sup>  | 2.00 <sup>a</sup>  | 2.08 <sup>a</sup>  | 2.11 <sup>a</sup>  | 0.01 |
| C22:6n3 (Decosahexeoic acid)        | 1.93 <sup>c</sup>  | 4.33 <sup>b</sup>  | 5.84 <sup>a</sup>  | 6.88 <sup>a</sup>  | 0.61 |
| TSFA <sup>1</sup>                   | 43.80 <sup>a</sup> | 39.80 <sup>b</sup> | 39.56 <sup>b</sup> | 38.18 <sup>b</sup> | 0.12 |
| TUFA <sup>2</sup>                   | 56.20 <sup>b</sup> | 60.20 <sup>a</sup> | 61.44 <sup>a</sup> | 62.82 <sup>a</sup> | 0.09 |
| MUFA <sup>3</sup>                   | 20.12 <sup>b</sup> | 22.10 <sup>a</sup> | 23.38 <sup>a</sup> | 24.22 <sup>a</sup> | 0.14 |
| PUFA <sup>4</sup>                   | 26.08 <sup>b</sup> | 40.10 <sup>a</sup> | 40.90 <sup>a</sup> | 41.60 <sup>a</sup> | 0.47 |

Means within a row with different letters were significantly different ( $P < 0.05$ ); <sup>1</sup>Total saturated fatty acid= C12:0 + C14:0 + C16:0 + C18:0 + C20:0 + C22:0; <sup>2</sup>Unsaturated fatty acid = (3 + 4); <sup>3</sup>Mono unsaturated fatty acid= C14:1C + C16:1c + C18:1c + C18:1n9t + C18:1n9c + C22:1; <sup>4</sup>Polyunsaturated fatty acid = C18:2 n6 + C20:5 n3 + C18:3n3 + C20:4n6 + C20:3n6 + C: 22:6n3



**Figure 1:** Fatty acid composition of thigh meat across the treatments

#### 4. Conclusion

Results from this study showed that rabbits fed TMSB had a better PUFA and MUFA level with a decrease in TSFA concentration. TMSB contains secondary compounds which exhibit many different biological properties

in animals including modulating the fatty acid composition of meat as well as promoting food safety. It can be concluded that feeding rabbits TMSB up to 9 mL per day had no negative effect on the general performance of animals.

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