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Research Article
Redox Potential of Pomegranate (*Punica granatum*) and Boldo (*Peumus boldus*) Included in the Diets of New Zealand White Rabbits

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Abstract

Background and Objective: Medicinal plants have been successfully utilized as an alternative treatment to synthetic substances. Now a days, continuous use of such synthetics have been found to be associated with adverse health problems. This study aimed to evaluate the redox potential of the ethanolic extract of pomegranate (*Punica granatum*) and/or boldo (*Peumus boldus*) included in the diets of growing white New Zealand male rabbits with the rate of 50 g kg^{-1}. **Materials and Methods:** Under intensive production system, rabbits were raised in batteries and randomly assigned into 8 groups (15 each). Treatments were control group G1 (basic diet without addition), G2 (Pomegranate peel), G3 (Pomegranate seeds), G4 (Pomegranate seeds+peel, 50:50%), G5 (boldo), G6 (G4+G5, 50:50), G7 (G4+G5, 75:25%, respectively) and G8 (G4+G5, 25:75%, respectively). Blood samples for biochemical analysis were withdrawn fortnightly in a month time following the adaptation period. The chemical composition of the tested plant extracts were measured and the total phenolic compounds were evaluated as gallic acid equivalent by one way analysis of variance (ANOVA). **Results:** Results from the current investigation revealed that pomegranate (peel and seeds) and boldo leaves were rich in protein, total lipids, total soluble carbohydrate, ash and phenolic contents. Moreover, for their redox potential and detoxification capacity, rabbit diet containing such materials maintained productive and physiological performance with low mortality rate. **Conclusion:** In the light of the present findings, it might be concluded that inclusion of such materials in feed for rabbits raised in batteries under intensive production system during growth is beneficial in terms of enhancing their redox potential with a consequent highly positive impact on production and health. Moreover, combination of both materials is positively encourage their use as herbal medicine.

Key words: Rabbits, *Punica granatum*, *Peumus boldus*, redox potential, blood biochemical, IgG, IgM, TNF-α

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Competing Interest: The authors has declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Under intensive production system, rabbits are reared in batteries. Rabbits as beings suffer from such cages (area, smell, surrounding micro-environment), also, the intensification of production could affect their physiological and hygienic status. Possible free radicals accumulation inside their bodies will increase the rate of cell death through necrotic and apoptotic actions[1].

Redox is the chemical reduction-oxidation reaction. A reduction reaction always occurs with an oxidation reaction. It is involved with electron transfer (reduction and oxidation). Oxidation is the loss of electrons or an increase in oxidation state by a molecule, atom or ion. Reduction is the gain of electrons or a decrease in oxidation state by a molecule, atom, or ion. Redox status of the body aims to remove free radicals from it. Free radicals can cause oxidative damage to all biomolecules and initiate a chain reaction, which results in physiological damage[2] that may lead to many degenerative diseases[3] as a result of accumulation of such compounds over a period of time. However, these effects are partially prevented by antioxidant compounds including α-tocopherol[4], silymarin[5] and salvianolic acid[6].

Pomegranate tree (*Punica granatum* L.) originated in the Middle East and India has been used for centuries in ancient cultures for its medicinal purpose. There are reports of the use of pomegranate as antiviral[7], antimicrobial[8] and anticancer agent[9]. Furthermore, it is widely acknowledged for antioxidant properties, which are higher quantities than in most other and/or fruit-related food items that were originally thought to contain the highest amounts of antioxidants[10]. High content of polyphenols was detected in the pomegranate fruits[11]. Boldo (*Peumus boldus*), the only species in the genus *Peumus* is tree of the family Monimiaceae natively endemic to the central region of Chile, occurring from 33-40° Southern latitude. Boldo has also been introduced to Europe and North Africa, though it is not often seen outside botanical gardens. Boldo leaves have a strong, woody and slightly bitter flavor and camphor-like aroma, are used for culinary purposes, primarily in Latin America. The leaves are used in a similar manner to bay leaves and also are used as an herbal tea, in Chile, Bolivia, Argentina, Paraguay, Peru, Uruguay, Brazil and bordering countries in South America. It is used as mild folk medicine in various South American countries in both urban and rural areas, even among people who do not usually drink herbal teas other than mate beverage. Boldo is officially listed as phytotherapic plant as cholagogue and choleretic, for treatment of mild dyspepsia in Brazilian pharmacopoeia[12]. On the other hand, the potential of boldo (*Peumus boldus*) as antioxidant food and the augmented effect between boldo and pomegranate (*Punica granatum* L.) in improving ROS system inside the rabbit body has not been investigated. Surai and Fisinin[2] documented the need to discover the antioxidant and detoxification capacity of the enormous vitagenes and vitamins in the several plants (either leaves or fruits peel or seeds). Furthermore, different plant parts should be investigated for their efficacy as antioxidants.

The aim of the present study was to investigate the redox potential of the inclusion of pomegranate (*Punica granatum* L.) and/or boldo (*Peumus boldus*) ethanolic extracts in rabbit diets as vitagenes to improve their growth and ameliorate any harmful effect of being in batteries under intensive production system and to use such plants as a nutrition and medicine.

MATERIALS AND METHODS

Experimental rabbits: A total of 120 male white New Zealand rabbits reared at Rabbit Production Unit, Faculty of Agriculture, Cairo University, Egypt were utilized in this experiment throughout the period from 10 January, 2015 to 15 August, 2015. Rabbits aging 6 months old were reared in metal batteries with automatic drinkers under intensive production system. During the treatment the average ambient temperature ranged from 18.5±0.11 to 22.05±0.32°C and relative humidity (%) ranged from 70.00±1.13 to 75.21±1.33.

Experimental basic ration without additions: Rabbits were fed a commercial diet for fattening according to NRC[13]. The chemical composition of this ration was estimated according to AOAC[14] and presented in Table 1. Water were offered *ad libitum*. Furthermore, vitamins and minerals (1 mL L^{-1} drinking water) were added.

Preparation of the examined plants samples: Samples of pomegranate were purchased from the local market in Egypt. Furthermore, samples of boldo (*Peumus boldus*) dried leaves were purchased from a commercial market at Sao Paolo, Brazil.

Table 1: Chemical analysis of the diets fed to the experimental rabbits

Chemical analysis	(%)
Moisture	14.21
Dry matter	85.10
Ash	0.65
Crude protein	16.23
Ether extract	3.54
Crude fiber	17.10
NFE	41.11

Vitamins and minerals per 1 mL diluted in 1 L drinking water contains: Vit. A 50000000 I.U, Vitamin D_3 5000000 I.U, Vitamin E 40000 mg, ascorbic acid 100000 mg, Mn 6000 mg, Zn 7200 mg, Fe 1500 mg, Cu 500 mg, I 120 mg, Se 100 mg, Co 100 mg, Mg 1000 mg, Na 14000 mg, K 7500 mg and P 10000 mg

Pomegranate (*Punica granatum*) samples were cut and edible parts were separated from the peel, each of two parts (seeds and peels) were dried in an air oven at 50°C till complete dryness. All samples were ground to fine powder preserved in plastic bags, put in a refrigerator till analysis or the use in the rabbit experiments.

Preparation of the ethanolic extracts: The dried pomegranate peel, pomegranate seed and boldo (*Peumus boldus*) were mixed with ethanol (80%) under shaking for 2 days. The resulting ethanolic extract was filtered and subsequently concentrated with a rotary evaporator under low temperature (40°C) and reduced pressure. The chemical analysis of the ethanolic extract of pomegranate peel, pomegranate seed and boldo (*Peumus boldus*) was determined according to AOAC[14].

Determination of the total phenols in the ethanolic extract: The total phenol content was determined according to the method of Oboh *et al.*[15] and Singleton *et al.*[16]. Mixing 0.5 mL of the pomegranate peel, pomegranate seed and boldo ethanolic extract of the fresh pomegranate peel, pomegranate seed and boldo (0.1 g dry weight/10 mL) with 2.5 mL 10% Folin–Ciocalteu reagent (v/v) and 2.0 mL of 7.5% sodium carbonate was subsequently added. The reaction mixture was incubated at 45°C for 40 min and the absorbance was measured at 765 nm in the spectrophotometer, gallic acid was used as standard phenol (3 μg mL^{-1}) and calculated as Eq. 1:

$$\text{Total phenols} = \frac{A_{\text{sample}}}{A_{\text{standard}}} \times \text{standard concentration} \quad (1)$$

Study design: After 4 weeks of the adaptation to the feeds, housing and surrounded environment, the experimental rabbits were randomly divided into 8 symmetric groups, each of 15 rabbits. The initial body weights of the different experimental groups are ranged from 1.490±1.35 to 1.560±0.85. The experimental groups were control group G1 (basic diet with no addition), G2 (Pomegranate peel), G3 (Pomegranate seeds), G4 (Pomegranate seeds+ peel, 50:50%), G5 (boldo), G6 (G4+G5, 50:50), G7 (G4+G5, 75: 25%, respectively) and G8 (G4+G5, 25: 75%, respectively). All the experimental rabbit groups had received the ethanolic extract of the investigated materials additions as 50 g extract for each kilogram of the feed except the control group.

Blood samples: Blood samples were taken by registered technician according the ethical standards from the marginal vein of the ears. The samples were taken by using a 21-gauge butterfly catheter and collected fortnightly into 5 mL heparinized tubes. Blood samples were centrifuged at room temperature using 3500 rpm for 15 min. The blood plasma was carefully withdrawn and kept in a deep freezer at -20°C pending carrying out different assays.

Table 2: Biochemical analysis of the blood

Analysis	Reference
AST	Reitman and Frankel[20]
ALT	Reitman and Frankel[20]
Alkaline phosphatase	Belfield and Goldberg[21]
Total bilirubin	Walters and Gerarde[22]
Plasma glucose	Trinder[23]
Urea	Fawcett and Scott[24]
Uric acid	Barham and Trinder[25]
Creatinine	Schirmeister *et al.*[26]
Total protein	Gornall *et al.*[27]
Albumin	Doumas *et al.*[28]
Superoxide dismutase	Nishikimi *et al.*[29]
Catalase	Aebi[30]
Glutathione reduced	Beutler *et al.*[31]
Lipid peroxide	Satoh[32]

Blood parameters: All the biochemical analysis of the blood plasma of the different experimental rabbits are presented in Table 2. The concentration of IGF-1 was estimated according to Breier *et al.*[17] using Bio Source® IGF-1-RIA-CT kit produced by BioSource Europe, Nivelles, Belgium. In this kit, a pre-treatment step is needed to dissociate the IGF-1 from its binding proteins (8 types) in order to improve the clinical performance of the assay because the binding proteins interfere with the radioimmunoassay for IGF-1. Cross-reactivity data provided by the manufacturer included values for IGF-1 (100%), IGF-II (0.7%), insulin (ND) and GH (ND). The coefficient of variability of intra-assay precision was less than 9.1% and less than 9.0% for inter-assay precision. A single radial immunodiffusion technique was developed to quantify blood plasma IgG using the immunoradiometric plates supplied by Biocientifica S.A., Argentina according to the method described by Fahey and McKelvey[18]. An immune-precipitation in agarose gel between the plasma IgG and anti-rabbit IgG antibody was formed, whereas the antigen diffuses radially out of the well into the surrounding gel-antibody mixture and proportionally related to the amount of IgG in plasma. The IgM in blood plasma is precipitated radially in an agarose gel containing an anti-rabbit IgM. The diameter of the expanded ring is related proportionally to the concentration of the antigen in blood. An enzyme linked immunosorbent assay was developed to quantify the tumor necrosis factor (TNF-α) using the kits from Orgenium Laboratories, Finland as described by Seriolo *et al.*[19].

Statistical analysis: Data was subjected to the one way analysis of variance using the general linear model of SAS[33]. The following statistical model was utilized:

$$Y_{ijk} = \mu + Tr_i + P_j + E_{ijk}$$

where, Y_{ij} is Observation measured, μ is Overall mean, Tr_i is Treatment (either i is G1 control group or i is G2 or i is G3 or i is G4 or i is G5 or i is G6 or i is G7 or i is G8 as in the experimental design), P_j is Periods (either j is 7th months old or j is 8th months old or i is 9th months old or i is 10th months old or i is 11th months old or i is 12th months old), E_{ijk} is experimental error assumed to be randomly distributed $(0, \sigma^2)$.

Statistical analysis system: The SAS/STAT statistics. Guide release, version 8.00 TS level OOMO, SAS Institute Inc., Cary, NC, USA[33] was used in this study.

RESULTS

Chemical composition of pomegranate peel, pomegranate seed and boldo: The chemical composition of the dried pomegranate seed and peel as well as dried boldo leaves shown in Table 3. It was observed that pomegranate (seed and peel) and boldo are rich in protein, elements and carbohydrates. The total soluble carbohydrates content was the highest in pomegranate seed and the lowest in boldo. The crude protein content in the examined plants showed higher value in boldo compared with pomegranate. Total lipids content was the highest in pomegranate peel and the lowest in pomegranate seeds. Their mineral content were ranged from 2.12-3.98%.

Total phenols and total antioxidant content: Total phenols of pomegranate seed and peel as well as boldo leaves crude ethanolic extract is given in Table 4. There was a high content of phenols. These explain the biological activities of pomegranate seed, pomegranate peel and boldo. Much of the total antioxidant activity of fruits and leaves is related to their phenolic content.

Productive performance traits of the experimental rabbits: Growth involves a complex set of metabolic events, which are genetically, hormonally and environmentally controlled. Environmental stressors like raising rabbits under the intensified production system could have an adverse effects on the growth performance of the rabbits especially the free radical formation and any disorders in the redox status inside the animal body.

Free radicals react with biological molecules and destroy the structure of cells, which eventually causes retarded growth, cancer, liver disease, aging, etc. The use of synthetic drugs has severe side effects. Therefore, it is useful to return to the natural antioxidants to overcome the side effects and detoxification of any harmful substance that may alter the partitioning of nutrients toward the growth and hence increase meat production.

Live body weight: Overall mean effect of the addition of the ethanolic extract of such examined plants to the diets of the experimental New Zealand white male rabbits was shown to be significant ($p<0.05$) either on the live body weight or in the growth rate (Table 5).

Feed consumption: Feed consumption (offered feeds–residual feeds) in the test day at the beginning of the experiment (at 7th months old) was almost similar in the experimental rabbits groups. Rabbits fed on the different experimental plants had consumed higher feed ($p<0.05$) throughout the entire experiment than that the control ones (Table 5). Total feed consumed by rabbits fed on the experimental diets containing either pomegranate or boldo or both of them (7-12 months of age) were higher than the control (Table 5).

Blood biochemical parameters

Liver function tests of the experimental rabbits: This study indicated that all the additions in this experiment did not affect the homeostatic reactions of the liver function. Treatments stimulated AST and ALT activities and bilirubin relative to those of control were mentioned in Table 6. Improvement in the activity of the two transaminases (AST and ALT) and blood bilirubin content were observed. In addition, ALP activity showed the same trend like that of transaminases activity under the same conditions (Table 6).

Table 3: Chemical composition (LSM±SE) of pomegranate peel, pomegranate seeds and boldo leaves

Samples	Moisture (%)	Total soluble carbohydrate (g/100 g)	Crude protein (g/100 g)	Total lipids (g/100 g)	Ash (g/100 g)
Pomegranate seeds	81.36±1.34	51.87±7.09	3.19±0.36	3.00±0.58	2.33±0.21
Pomegranate peel	75.11±2.06	48.23±5.95	4.13±0.50	9.00±1.15	3.98±0.47
Boldo leaves	89.97±0.88	19.18±2.19	5.03±0.18	6.33±0.33	2.12±0.33

Data are expressed as Mean±SE and calculated as a percent of dry weight

Kidney function tests of the experimental rabbits: The kidneys function tests are within the normal physiological range in all rabbits as presented in Table 7. This indicated that all the additions in this experiment did not exert any burden effect on the kidney function.

The data showed a decrease in urea, uric acid and creatinine in the rabbit consumed the examined additions, also the augmented effects of the addition of boldo and pomegranate at any ratio was documents.

Antioxidants biomarkers in blood plasma of the experimental rabbits: Antioxidant and biological activities of pomegranate peel, seed and boldo leaves extracts are explained from their high content of total phenols (Table 4). Boldo leaves and pomegranates contain numerous phytochemicals, which are potent antioxidant compounds found naturally in plants that help to prevent and treat disease. The antioxidants in boldo leaves can help to reduce free radical-induced damage to the cells and DNA. The examined antioxidant biomarkers showed a significant increase ($p<0.05$) in the activities of catalase and SOD (Table 8).

Table 4: Total amount of plant phenols compounds of the ethanolic extract of pomegranate seed, pomegranate peel and boldo

Samples	Total phenols*
Pomegranate seeds	14.26±0.36
Pomegranate peel	22.73±0.54
Boldo leaves	9.66±0.34

Data are expressed as Mean±SE. *mg g^{-1} plant ethanolic extract as gallic acid equivalent

Blood plasma proteins and IGF-1: Plasma insulin like growth factor-1 is the mediator of growth hormone action and its concentrations is affected by any physiological disorders that could be happened by the exposure to any physiological stressors. The average concentrations of blood plasma IGF-1, total protein, albumin, globulin and albumin globulin ratio for each of the experimental groups is shown in Table 9. Blood plasma IGF-1 in New Zealand white male rabbits

Table 5: Productive performance of the experimental growing New Zealand white male rabbits

Groups	LBW at 7th months	LBW at 12th months	DWG	MR (%)	Feed consumption (g day^{-1})	Total feed consumed (g)
G1	910.6±90.4	1810.0±19.9^{b}	25.6	11	100.40±1.09	6940.21
G2	897.0±81.7	1785.7±71.5^{b}	21.1	14	129.45±1.16	7112.63
G3	933.5±44.9	1770.0±32.1^{a}	20.5	15	113.00±0.08	7512.92
G4	857.0±21.5	1690.0±21.6^{a}	26.1	12	112.20±0.01	6940.21
G5	910.5±14.5	1590.0±82.6^{a}	23.2	13	106.10±0.09	6890.11
G6	877.0±11.7	1820.0±19.9^{b}	26.0	10	124.11±1.20	8002.13
G7	905.5±14.9	1850.0±14.6^{b}	25.9	9	125.21±0.67	7812.15
G8	839.0±41.7	1910.0±12.9^{b}	27.1	8	130.05±0.66	8119.11

Data are expressed as Mean±SE. LBW: Live body weight (g), DWG: Daily weight gain from 7-12 months (g day^{-1}), MR: Mortality rate. Within the same column, LSM having different superscripts differ at $p<0.05$

Table 6: Liver function tests of the experimental rabbits

Groups	AST (U L^{-1})	ALT (U L^{-1})	AST/ALT ratio	Alkaline phosphatase (IU L^{-1})	Bilirubin (mg dL^{-1})
G1	133.16±7.19^{bc}	52.41±3.56^{b}	2.60^{c}	30.63±1.23^{ab}	0.14±0.02^{bc}
G2	182.39±8.47^{a}	76.71±0.86^{a}	2.38^{c}	31.87±2.83^{a}	0.26±0.04^{a}
G3	157.64±19.59^{ab}	54.72±1.80^{b}	2.87^{ab}	30.69±2.37^{ab}	0.21±0.02^{ab}
G4	134.98±9.94^{bc}	45.40±3.05^{bc}	3.02^{ab}	24.20±3.33^{ab}	0.16±0.03^{bc}
G5	125.72±5.16^{c}	34.10±2.20^{d}	3.73^{ab}	23.98±0.38^{bc}	0.12±0.02^{c}
G6	126.34±5.65^{c}	48.87±3.07^{bc}	2.64^{c}	29.51±1.49^{ab}	0.17±0.03^{bc}
G7	147.27±2.21^{bc}	40.38±4.30^{cd}	3.81^{a}	23.12±1.74^{bc}	0.16±0.01^{bc}
G8	141.74±8.55^{bc}	51.29±6.15^{bc}	3.01^{ab}	22.21±3.38^{c}	0.18±0.01^{bc}

Each value represents the mean of 15 rabbits (Mean±SE). Within the same column, LSM having different superscripts differ at $p<0.05$

Table 7: Kidneys function (mg dL^{-1}) of the experimental rabbits

Groups	Urea	Uric acid	Creatinine
G1	23.96±2.04^{b}	0.52±0.07^{b}	0.47±0.01^{ab}
G2	31.60±2.73^{a}	0.75±0.06^{a}	0.59±0.06^{a}
G3	19.77±0.34^{c}	0.60±0.04^{ab}	0.56±0.01^{a}
G4	20.06±0.99^{c}	0.46±0.10^{b}	0.53±0.06^{a}
G5	16.83±0.59^{cd}	0.50±0.06^{b}	0.37±0.03^{b}
G6	19.87±0.98^{c}	0.44±0.04^{b}	0.47±0.02^{ab}
G7	16.57±0.58^{cd}	0.57±0.04^{b}	0.47±0.05^{ab}
G8	14.22±0.58^{d}	0.46±0.05^{b}	0.51±0.10^{ab}

Each value represents the mean of 15 rabbits (Mean±SE). Within the same column, LSM having different superscripts differ at $p<0.05$

Table 8: Antioxidants biomarkers in blood plasma of the experimental rabbits

Groups	SOD (U g^{-1})	Catalase (U g^{-1})	GSH (mg g^{-1})	Lipid peroxide (nmol g^{-1})
G1	545.49±12.60^{e}	70.86±2.00^{a}	7.79±1.39^{bc}	12.60±0.80^{a}
G2	445.49±27.70^{e}	91.34±1.92^{bc}	14.50±0.86^{a}	7.83±0.22^{c}
G3	930.85±26.66^{b}	47.22 ±2.19^{d}	10.74±0.96^{b}	10.89±0.83^{b}
G4	1205.55±43.38^{a}	47.22 ±2.19^{d}	3.86±0.24^{d}	8.83±0.62^{c}
G5	678.12±38.06^{cd}	88.44±2.04^{c}	8.71±0.63^{bc}	11.17±.88^{b}
G6	640.12±28.84^{d}	99.30±4.98^{b}	14.50±0.86^{a}	5.75±0.30^{d}
G7	600.49±41.49^{d}	85.44±1.04^{c}	8.71±0.63^{bc}	8.83±0.62^{c}
G8	771.50±65.09^{c}	95.80±4.74^{bc}	6.85±0.32^{c}	5.19±0.38^{d}

Each value represents the mean of 15 rabbits (Mean±SE). Within the same column, LSM having different superscripts differ at p<0.05

Table 9: Concentrations (LSM±SE) of blood plasma IGF-I, total proteins, albumin, globulin and albumin/globulin ratio of the experimental rabbits

Groups	IGF-I (ng mL^{-1})	Total protein (g dL^{-1})	Albumin (g dL^{-1})	Globulin (g dL^{-1})	Albumin/globulin ratio
G1	159.7±93.3^{c}	7.13 ±0.35^{c}	3.21±0.22^{b}	3.92±0.22^{b}	0.82
G2	209.0±51.2^{b}	8.24 ±0.30^{b}	3.80±0.40^{a}	4.44±0.40^{a}	0.86
G3	305.4±15.1^{a}	9.51±0.24^{a}	4.65±0.11^{a}	4.86±0.11^{a}	0.96
G4	185.4±75.6	8.44±0.30^{b}	3.80±0.40^{a}	4.64±0.40^{a}	0.86
G5	229.0±51.2^{b}	9.44±0.30^{b}	3.80±0.40^{a}	5.44±0.40^{a}	0.80
G6	249.0±31.2^{b}	8.51±0.24^{a}	3.65±0.11^{a}	4.86±0.11^{a}	0.92
G7	295.2±29.1^{a}	9.51±0.24^{a}	4.65±0.11^{a}	4.86±0.11^{a}	0.96
G8	355.4±18.6^{a}	10.51±0.24^{a}	5.65±0.11^{a}	4.86±0.11^{a}	0.96

Data are, expressed as Mean±SE. Within the same column, LSM having different superscripts differ at p<0.05

Table 10: Concentrations (LSM±SE) of blood plasma IgG, IgM and TNF-α of the experimental rabbits

Groups	IgG (mg dL^{-1})	IgM (mg dL^{-1})	TNF-α (pg dL^{-1})
G1	214±77^{c}	17.83±4.1^{b}	5.2±6.4^{b}
G2	310±25^{b}	23.6±3.4^{a}	1.55±4.8^{a}
G3	376±17^{a}	27.8±2.8^{a}	1.48±2.9^{a}
G4	274±12^{c}	27.1±2.1^{b}	1.75±1.2^{a}
G5	256±43^{c}	25.1±2.2^{a}	1.18±2.3^{a}
G6	356±14^{a}	30.8±2.4^{a}	2.09±0.9^{a}
G7	326±12^{a}	22.8±2.8^{a}	1.55±4.8^{a}
G8	396±45^{a}	21.1±0.5^{a}	2.55±2.1^{a}

Data are expressed as Mean±SE. Within the same column, LSM having different superscripts differ at p<0.05

consumed in their diets pomegranate and/or boldo was significantly increased (p<0.05) than the controls (Table 9). The values of blood plasma IGF-1 in the experimental rabbits consumed such dietary additions could explain the a beneficial effect on the liver function (main source of IGF-1) and the increase in the growth rate and other productive traits as shown in Table 5. Blood plasma proteins of the experimental rabbits (Table 9) are within the normal physiological range.

Plasma IgG (mg dL^{-1}), IgM (mg dL^{-1}) and TNF-α (pg mL^{-1}): Plasma concentrations of the immunological fractions IgG and IgM and the lymphoid factor (TNF-α) are presented in Table 10. Rabbits consumed diets supplemented with pomegranate and/or boldo had higher (p<0.05) plasma IgG and lower (p<0.05) plasma TNF-α than control rabbits. The highest IgG value (396 mg dL^{-1}) was recorded for rabbits in G8. Also, control rabbits fed on the basic ration had the lowest value (p<0.05) of IgM (17.83 mg dL^{-1}) in the blood plasma. The plasma concentration of TNF-α were the highest (p<0.05) in the control rabbits fed on the basic ration without any addition. In addition, pomegranate (peel and/or seeds) and boldo ethanolic extract addition to the diets of the rabbit improve their immunological responses and reduce the lymphoid factor (TNF-α).

DISCUSSION

Rabbits reared in batteries are susceptible to free radical formation inside their body, hence face morbidity, lower growth performance and mortality. Also, under the intensification of production vitamins, drugs, minerals consumption are increased by rabbits, hence the need of adding vitagenes is increased. The present research aimed to examine *in vivo* the redox potential of pomegranate seeds, peel and/or boldo as an untraditional feed supplementation and to examine their benefits during the growth period of rabbits and as herbal medicine, hence return to the nature to overcome the side effects and accumulation of the drugs.

The results revealed that pomegranate (seeds and peel) and boldo are rich in protein, minerals and carbohydrates, hence it is possible to use it as an untraditional feed. Results from this study revealed that boldo leaves had high crude protein and low total soluble carbohydrates and the pomegranate seeds were higher in total soluble carbohydrates and lower in crude protein and total lipids than the pomegranate peel. On the other hand, the total phenols content in such examined plants proved it is possible use as an antioxidant and explain their beneficial health effects by potential in free radicals removals. Flavonoids are potent antioxidants which mediated by chelating metallic ions by their hydroxyl group[34,35]. Natural polyphenols have beneficial health effects by their antioxidant activity since such compounds are capable of removing free radicals, chelate metal catalysts, activate antioxidant enzymes, reduce α-tocopherol radicals and inhibit oxidases[36-39] attributed the biological activity of some herbal plants to the presence of the polyphenols. Lower mortality rates and better production performance in the rabbit groups fed diets containing such examined plants document their capability as functional feeds. Furthermore, the examined liver and kidney function tests showed no harm effects on the rabbits consumed such plants. All the activities of liver enzymes and kidney function tests were in the normal physiological range reported by Rahmat *et al.*[40]. The examined antioxidant biomarkers showed increase in the activities of catalase and SOD in blood plasma of rabbits fed diets contain pomegranate (peel and/or seeds) and/or boldo (leaves) which attributed to the increase in the hepatocellular activities in these rabbits in compare to the control. In agreement with and Surai and Fisinin[2] and HMPC[12]. The data of the examined endogenous antioxidant biomarkers (SOD, catalase, GSH and lipid peroxide) showed documented physiological benefits of adding pomegranate and/or boldo in the diets. The normal blood plasma proteins in rabbits fed diets containing boldo and/or pomegranate reflects the beneficial additions of such material to improve the liver function to produce plasma proteins. Also, higher blood plasma protein fractions and IGF-1 in rabbits fed diets containing pomegranate (peel and/or seeds) and/or boldo (leaves) than control rabbits declare a better liver function and explain the better growth performance. Growth hormone (GH) acts to control IGF-1 plasma levels and IGF-1 acts directly at the cellular level. In addition, serum IGF-1 levels were positively correlated with weight and positive energy balance[24,41]. In addition, pomegranate (peel and/or seeds) and boldo ethanolic extract addition to the diets of the rabbit improve their immunological responses and reduce the lymphoid factor (TNF-α). High levels of TNF-α had been reported with various pathological conditions include septic shock, autoimmune diseases, hepatitis, leukemia, multiple sclerosis, rheumatoid arthritis and septicemia[19,42]. Boldo has been shown to exert anti-inflammatory and anti-pyretic (anti-fever) effects. Boldo is an effective inhibitor or prostaglandin synthesis, part of the inflammatory process[12]. The augmented effects of the presence of the ethanolic extract of examined plans together in the diets of the rabbits are documented, rabbits in G8 had the lowest mortality rate (8%) and the highest daily weight gain (27.1 g). The study revealed the possibility of using pomegranate (peel and/or seeds) and/or boldo (leaves) in the diets of rabbits and gaining their nutritional and functional capabilities. Otuechere *et al.*[43] reported that normal blood biochemistry profiles are good indicator to the tissue integrity, that may contain antioxidant compounds, against exposure to any toxic materials contain. These results are in agreement with different studies[40,44,45] which ameliorated the harmful effect of CCl_4 that causes liver damage by using an antioxidant plants. The HMPC[12] documented the detoxification effect of boldo extracts and the choleretic properties (stimulating bile flow).

CONCLUSION AND FUTURE RECOMMENDATIONS

The information presented in this study was trial investigations that shown some redox potential inside the rabbit bodies and improvement in the liver function without any effect on their kidney after addition of pomegranate (peel and/or seeds) and boldo (leaves) extract to the diets of growing male New Zealand white rabbits. In addition, this antioxidant function of these additions improve the productive performance, immunological status and reduce the lymphoid factor TNF-α. The augmented effect of adding pomegranate (peel and/or seeds) and/or boldo (leaves) to the diets was also documented for all studied rates.

SIGNIFICANCE STATEMENTS

The current investigation has provided a piece of evidence regarding the positive influence of pomegranate (*Punica granatum*), boldo (*Peumus boldus*), involved in the diets of the white New Zealand male rabbits, which have been shown to enhance the productive performance with low mortality rate. Furthermore, biochemical analysis indicated normal physiological behavior and better redox potential and detoxification capacity for tested material. The current investigation might encourage and open new avenues to researcher to determine the most adequate combinations to achieve better production and health levels.

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