



## Full Length Article

# Effects of manganese and boron levels in breeder Japanese quails ration on performance, eggshell quality, and bone biomechanical properties

Muhammet Ali Kara

Siirt University, Department of Animal Science, Siirt, Turkey



## ARTICLE INFO

## Keywords:

Japanese quail  
Manganese  
Boron  
Eggshell quality  
Bone characteristics

## ABSTRACT

**Background:** Improved understanding of the impact of (Mn) and boron (B) levels in the dietary regimen of Japanese quails on their performance, eggshell quality, and bone properties might facilitate the optimization of their nutritional intake and improving health and productivity.

**Methods:** The effects of Mn and B added to the rations of breeder Japanese quail alone or together were investigated on performance, eggshell quality, and bone biomechanical characteristics.

**Results:** Egg production, egg mass, feed consumption, and feed conversion ratio values related to the performance of breeding quail were not affected by the single or combined addition of Mn and B. Average live weight gain and egg weight values were significantly affected by Mn and B additions. The highest average body weight gain (47.917 g) and egg weight (13.805 g) values were reached at 120 × 20 (120 mg Mn/kg + 20 mg B/kg) interaction. The interaction of 120 × 20 was appropriate considering the eggshell ratio and eggshell fracture strength values. The ultimate shear force and shear stress parameters were significantly affected by 60 mg/kg Mn or 20–80 mg/kg B additions compared to the control. The addition of 40–80 mg/kg B significantly increased the shear fracture energy. The femur bone Iron (Fe) content significantly increased with the combined additions of Mn and B. The combined Mn and B additions had no significant effect on bone calcium (Ca), phosphorus (P), and magnesium (Mg) contents, while the 0Mn × 20B interaction resulted in significantly higher bone Ca and P content than the control. The increase in Fe, Ca, P, and Mg accumulation in the bones could be associated with B addition in the Mn × B interaction.

**Conclusion:** In the production of breeding Japanese quail, the addition of 120 mg Mn/kg to the diet would be appropriate, considering the performance characteristics of the quails, when Mn is used alone. The results revealed that the addition of 60 × 120 (Mn × B) interaction can be recommended for successful Japanese quail production. Nevertheless, more detailed studies assessing the effect of combined Mn and B additions to Japanese quail diets are needed to elucidate the action mechanism and to draw more precise conclusions.

## 1. Introduction

Japanese quails (*Coturnix japonica*) are increasingly attracting attention for egg or meat production in many countries in Asia, Europe, the Middle East, and America (Narinç et al., 2013). In Japan, commercial quail farms primarily focus on achieving high egg output, but in other countries such as Spain and France, there is a greater emphasis on meat production (Minvielle, 1998). In Turkey, the rearing of Japanese quails mostly occurs within family-operated, small-scale operations, with the dual purpose of meat and egg production (Narinç et al., 2013).

The high breast meat yield, high nutritional value, and delicious taste of quail meat are among the main factors why quail meat is popular in Turkey (Gözet and Baylan, 2020). The quail eggs, which are preferred

due to their high nutritional value, are believed to have a positive impact on the physical and mental development of children and also strengthen the immune systems. In addition, eggs are considered to have a supportive role in the therapy of various diseases such as upper respiratory tract infections, cancer, tuberculosis, asthma, anemia, stomach, liver, and kidney disorders, etc. Therefore the demand for quail meat and eggs is high in the market (Sayılı et al., 2014). Quail breeding is widely spread in Turkey since quail breeding does not require a large area and high investment, and high productivity can be achieved in a short time following the establishment of the enterprise (İpek et al., 2002).

The performance characteristics of Japanese quails, such as egg and meat yield, have been significantly improved by selection (Tüzün and Aktan, 2012; Narinç et al., 2016). Improving managerial and

E-mail address: [muhammetalikara@siirt.edu.tr](mailto:muhammetalikara@siirt.edu.tr).

<https://doi.org/10.1016/j.jksus.2024.103113>

Received 2 August 2023; Received in revised form 31 October 2023; Accepted 24 January 2024

Available online 26 January 2024

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environmental conditions along with selection and breeding can also contribute to improving production (Chahil et al., 1975; Hussain et al., 2016). Previous studies have shown that feed cost accounts for about 70–80 % of the total production cost of Japanese quail and are primarily responsible for the quality of the final product (Cruz and Fernandez, 2011; Çimrin and Tunca, 2012). Feed additives play an essential role in poultry nutrition to support animal performance and welfare due to their contribution to feed quality in poultry nutrition (Budağ et al., 2018). Trace minerals are particularly emphasized as a feed additive since they are involved in many functions such as skeletal development, growth, expansion of skin and other epithelial tissues, immune system functions, healing of wounds, appetite, reproduction, metalloenzymes, and protein synthesis, stabilization of cell membranes, the composition of DNA and RNA, etc. (Underwood and Suttle, 1999). Trace minerals as well as macro minerals have been recommended as a supplement to the ration to effectively meet the nutritional demands of Japanese quail (Çimrin and Tunca, 2012).

The need for manganese (Mn), an essential trace element, has recently increased essential trace element has increased due to the increased performance capabilities of birds (Olgun, 2017). The Mn activates the hydrolase and kinase group enzymes and is also a component of arginase, pyruvate carboxylase, and superoxide mutase enzymes (Arıcı et al., 2019). Mn has a very important role in the maintenance of growth and performance, matrix formation and bone development, optimal eggshell quality, and prevention of perosis, since Mn is involved in carbohydrate, amino acid, and lipid metabolism (Palacios, 2006; Dermience et al., 2015; Olgun, 2017; Arıcı et al., 2019). Therefore, Mn-deficient breeder rations cause chondrodystrophy in chick embryos (Leeson, 2022).

The mechanism of boron (B) action has not been fully elucidated yet. The studies have demonstrated that B interacts with other nutrients and their metabolisms (such as Ca, P and Mg), and accordingly plays a regulatory role in bone metabolism (Naghii and Samman, 1993; Armstrong, 2000). Boron is essential for preventing nutritional disorders, osteoporosis, and low immune function (Abdelnour et al., 2018). Boron is also believed to promote hormone regulation and brain functions (Khaliq et al., 2018; Abdelnour et al., 2018). Thus B addition to laying hens ratios increased bone fracture strength prevented joint disorders, and improved egg quality (Mizrak et al., 2008; Koçbeker et al., 2017). Similarly, B addition (20 mg/kg) to the ratios of Japanese quails increased eggshell quality and performance (Kara, 2022). Therefore boron is required in animals when they need to respond to nutritional stress that adversely affects calcium metabolism, including magnesium deficiency (Nielsen, 1990).

This study aims to reveal the effects of different manganese and boron ratios added to the breeding quail (*Coturnix coturnix japonica*) rations on performance, eggshell quality, and bone biomechanical properties.

## 2. Materials and Methods

### 2.1. Materials

A sufficient number (800–1000) eggs were collected from the breeding flock and incubated. The hatched chicks were grown for six weeks and weighed. The quails of similar weight were selected and used in the experiment. A total of 270 breeding quails (7 weeks old) were used in the experiment. Nutrient compositions of raw materials used in the basal ration were given in Table 1.

### 2.2. Methods

In the experiment, 0, 20, 40, 80, and 160 mg/kg boron (B) were added to the feed containing 0, 60, and 120 mg/kg manganese (Mn). Accordingly, 15 different rations were used between the 7 and 28 weeks stage of the quails. The Mn was added to the rations as manganese sulfate and the B was as boric acid. All the rations, except Mn were prepared to contain nutrient levels recommended by the NRC (1994) for breeding quail (Table 2). The experiment was carried out with three replications in a 3x5 factorial design consisting of 3 Mn and 5B levels. The experiment included a total of 45 subgroups. Every cage cell was considered a replicate, and 4 female + 2 male quails were placed in each cell. Consequently, a total of 270 animals were used in the experiment.

### 2.3. Performance characteristics

The live weights of quails were determined by group weighing at the beginning and end of the experiment. The feed consumption of each bird group was determined for 28-day periods, and the daily average feed consumption was calculated. Eggs were collected and recorded daily, and egg yields were calculated using these records. The egg weights were determined by weighing all eggs collected in the last three days of the 28 days on a 0.1 g precision scale. Egg mass was calculated by multiplying the average egg production per unit (amount/day) by the average egg weight using the following equation (Eq. 1).

$$\text{Egg mass (g)} = \frac{\text{Total number of eggs}}{\text{Number of birds in the cell}} \times \text{average egg weight} \quad (1).$$

The average daily feed consumption per quail was divided by egg mass to calculate the feed evaluation coefficient.

### 2.4. Eggshell characteristics

Egg-specific gravity, shell fracture strength, and shell weight values were determined by using ten randomly selected eggs from each group in the last three days of the 28 days. Egg-specific gravity was measured by weighing selected eggs in air and water using an appropriate mechanism placed on the scale. The weight of the selected eggs in air and water was measured using a suitable mechanism placed on the scale, and then egg-specific gravity was determined using the principle of Archimedes (Eq. 2) (Wells, 1968).

$$\text{Egg specific gravity (g/cm}^3\text{)} = \frac{\text{Weight of eggs in air}}{\text{Difference between egg weights in air and water}} \quad (2).$$

**Table 1**

Nutrient composition of raw materials used in the ration.

Raw materials	HP (%)	ME (kcal/kg)	Ca (%)	Total P (%)	Lysine (%)	Methionine (%)	Cystine (%)
Barley	11.00	2761	0.07	0.40	0.20	0.20	0.24
Corn	8.80	3300	0.03	0.27	0.20	0.18	0.15
Soybean meal	47.03*	2254	0.28	0.66	2.70	0.69	0.67
Sunflower seed meal	36.02*	2018	0.40	1	1.28	0.60	0.56
Vegetable oil	–	9000	–	–	–	–	–
Marble powder	–	–	37.50	–	–	–	–
DCP	–	–	24	18	–	–	–

\*Determined using the result of the analysis.

**Table 2**

Raw material and calculated nutrient composition of the basal diet used in the experiment.

Raw materials	%
Barley	7.20
Corn	44.30
Soybean meal	28.60
Sunflower seed meal	6.00
Fat	5.30
Calcium oxide	6.20
DCP	1.40
Salt	0.40
L-Lysine	0.10
DL-methionine	0.15
Vitamin Premix <sup>1</sup>	0.25
Mineral Premix <sup>2</sup>	0.10
Total	100
Nutrient composition	
Energy (kcal ME/kg)	2903.30
HP (%)	20.03
Ca (%)	2.78
Ca (%*)	2.74
Total P (%*)	0.71
Usable P (%)	0.39
Lysine (%)	1.05
Methionine (%)	0.48
Methionine + Cystine (%)	0.79
Boron (mg/kg*)	14.18
Zinc (mg/kg*)	33.58

\*ICP analysis results.

<sup>1</sup> Vitamin premix in 1 kg of the ration provides: vitamin A, 8800 IU; vitamin D3, 2200 IU; vitamin E, 11 mg; nicotinic acid, 44 mg; Cal-D-Pant., 8.8 mg; riboflavin 4.4 mg; thiamine, 25 mg; vitamin B12, 6.6 mg; folic acid, 1 mg; D-Biotin, 0.11 mg; choline, 220 mg.

<sup>2</sup> Mineral premix (Mn free) 1 kg of the ration provides: iron, 60 mg; copper, 5 mg; iodine, 1 mg; cobalt, 0.2 mg; selenium, 0.15 mg.

The shell fracture strengths of the eggs of which specific gravity was determined, were measured using a fracture test device (Egg Force Reader, Orka Food Technology). The shells of these eggs were separated, washed with tap water, dried at room temperature for three days, and then weighed by the precision scale to determine shell weight. The following equation was used to calculate the shell ratio of eggs (Eq. 3).

$$\text{Shell rate (\%)} = \frac{\text{Shell weight}}{\text{egg weight}} \times 100 \quad (3).$$

### 2.5. Bone characteristics

All female birds in each subgroup were slaughtered and cleaned to determine the bone mineral content and biomechanical properties, the right and left tibia and femurs were taken, bagged, and stored in a deep freezer at  $-20^{\circ}\text{C}$  until the analysis. The right tibia was used to determine bone biomechanical properties, while the right femur bone was used for mineral content. The frozen bones were kept at room temperature until thawed, then placed in hot water for 3 min. The soft tissues of the bones were removed. The bones were then oven-dried at  $105^{\circ}\text{C}$  for 24 h. The dried bones were crushed and the subsamples were placed in porcelain crucibles and burned in a muffle furnace at  $600^{\circ}\text{C}$  for 24 h. The ash content of the bones was determined and the percent ash values were calculated. A sample of 0.2–0.3 g was taken from the ash and placed in Teflon containers, and 10 ml of 65 % nitric acid was added. The sample was burned in a microwave device (Cem-Mars 5) at 175 PSI and  $180^{\circ}\text{C}$  for 30 min, filtered through filter paper, and made up to 25 ml. Elemental components of the sample were determined using an ICP-AES instrument (Atomic Emission Spectrometer) (Inductively Coupled Plasma Atomic Emission Spectrometer, Varian-Vista Model).

Bone dimensions and tests of bone mechanical properties (ultimate shear force, shear stress, and shear fracture energy) were measured

using the right fresh tibia bones. The soft tissues were removed from the bones, and the outer diameters (tibia thickness) were measured at the center of the diaphysis. The bones were rotated during measurement, and their narrow and wide edges were measured using a digital caliper. The average of these two values was used as the bone thickness. The ultimate shear force of the bones of which the thicknesses measured was determined in the tensile strength tester using advanced fulcrum prepared according to the S459 DEC 01 standard of ANSI/ASAE. The loading speed of the device was set as 5 mm/min, and the cutting test was performed after the proper installation of the bone to the device.

The shear force was performed on a 5.64 mm section of the center of the bone. The data of the shear force and shear force–deformation diagrams were obtained at the end of the test. Shear fracture energy was calculated using these data. The broken or cut bones were cleaned, and the wall thickness of the bones was measured using a digital caliper. Bone cross-sectional area was calculated from the tibia (external) diameter and wall thicknesses. Shear stress was calculated by dividing the bone shear force into the cross-sectional area.

### 2.6. Statistical analysis

The experiment was carried out in a factorial experiment design (3 Mn and 5B levels = 15 factors) with three replications. Analysis of variance (ANOVA) was used to data to determine the effects of the treatments on the performance parameters using Minitab (2000) statistical package program. Normality was tested in the data prior to ANOVA, which indicated that the data had normal distribution. When the ANOVA indicated a statistically significant effect, Duncan's multiple comparisons test was used to compare the means.

## 3. Results

### 3.1. Performance characteristics

Table 3 presents the impact of Mn or B supplementation at varying levels in the rations, as well as the interactions between Mn and B, on the performance of quails. The findings of the study suggest that the inclusion of Mn in the diet led to a marginal improvement in egg production, egg mass, feed intake, and feed conversion ratio. Nevertheless, this increase was not statistically significant. The average weight gain exhibited a significant increase from 27.933 g to 35.283 g upon the use of 120 mg Mn/kg. The incorporation of 60 mg Mn/kg resulted in a significant reduction in egg weight as compared to the control group (0 mg Mn/kg). However, the addition of 120 mg Mn/kg led to an increase in egg weight. The egg weights were 12.861, 12.667, and 13.412, respectively, upon the addition of 0, 60, and 120 mg Mn/kg to the rations.

Except for the average weight gain, the measured variables did not exhibit a statistically significant impact on the outcome of the B addition into the diets. The study's greatest mean weight gain was recorded for the use of 20 mg/kg of supplemental B, which corresponded to 37.111 g, in comparison to the control group. Furthermore, the weight gain exhibited a declining trend as the amount of B increased.

Consistent with the findings of the individual Mn or B addition, the study revealed that the combined effect of Mn and B did not result in significant differences in egg production, egg mass, feed consumption, and feed conversion ratio. However, the addition of Mn and B (Mn  $\times$  B interaction) at varying dosages had a significant impact on both the average body weight gain and egg weight values. The incorporation of 120 mg Mn/kg and 20 mg B/kg (120  $\times$  20) into the diets resulted in obtaining the greatest mean body weight gain and egg weight values. The maximum mean increase in body weight and egg weight were recorded as 47.917 g and 13.805 g, correspondingly. Furthermore, there was a reduction in both overall body weight gain and egg weight values in relation to the varying levels of Mn or B used in the interactions, as indicated in Table 3.

**Table 3**

Effects of different levels of manganese and boron supplementation to rations on breeder quail performance.

Mn/B levels (mg/kg)	Average body weight gain (g)	Egg Production (%)	Egg weight (g)	Egg mass (g/quail/day)	Feed intake (g/quail/day)	Feed conversion ratio (g feed/g egg)
<b>Mn levels</b>						
0	27.93b	81.51	12.86b	10.38	30.11	2.92
60	30.27b	81.26	12.66c	10.40	31.03	2.89
120	35.28a	83.56	13.41a	10.96	30.27	2.69
SEM	0.83	2.09	0.05	0.26	0.46	0.08
P-value	<0.0001	0.69	<0.0001	0.22	0.34	0.11
<b>B levels</b>						
0	28.19b	78.25	12.77	10.01	31.01	3.01
20	37.11a	81.70	13.13	10.73	30.84	2.78
40	27.87b	85.13	12.99	10.92	30.62	2.74
80	34.33a	82.04	12.97	10.50	29.57	2.82
160	28.30b	83.41	13.01	10.74	30.30	2.80
SEM	1.08	2.70	0.079	0.34	0.60	0.10
P-value	<0.0001	0.48	0.0572	0.40	0.48	0.44
<b>MnxB interaction</b>						
0x0	25.00fgh	76.66	12.78de	9.80	31.44	3.12
0x20	40.83b	81.78	12.71def	10.58	32.08	2.90
0x40	20.33 h	86.78	12.88 cd	10.81	30.99	2.81
0x80	30.25ef	81.48	13.03 cd	10.30	27.67	2.90
0x160	23.25gh	80.83	12.89 cd	10.42	28.36	2.87
60x0	34.91cde	74.88	12.79de	9.77	30.26	3.10
60x20	22.58gh	82.38	12.89 cd	10.63	30.98	2.79
60x40	26.44 fg	84.10	12.84 cd	10.93	31.01	2.78
60x80	32.75de	81.96	12.36f	10.31	31.29	2.85
60x160	34.66cde	82.97	12.44ef	10.35	31.60	2.92
120x0	24.66gh	83.21	12.75def	10.47	31.33	2.82
120x20	47.91a	80.95	13.80a	10.99	29.46	2.64
120x40	36.83bcd	84.52	13.26bc	11.01	29.87	2.63
120x80	40.00bc	82.68	13.51ab	10.89	29.75	2.72
120x160	27.00 fg	86.43	13.72a	11.46	30.95	2.61
SEM	1.87	4.69	0.11	0.59	1.05	0.18
P-value	<0.0001	0.9763	0.001	0.99	0.13	0.99

### 3.2. Eggshell characteristics

Table 4 presents the parameters pertaining to the impact of Mn and B supplementation, either individually or in combination, on eggshell quality. With the exception of the egg surface area, all parameters related to eggshell quality were significantly affected ( $P \leq 0.01$ ) by the level of Mn introduced into the diet. Although the inclusion of 60 mg Mn/kg resulted in a small increase in egg-specific gravity, the addition of 120 mg Mn/kg led to a significant reduction in this parameter. The egg-specific gravity exhibited was 1.0715 g/cm<sup>3</sup> in the absence of Mn (control), whereas it was 1.0704 g/cm<sup>3</sup> with the addition of 120 mg Mn/kg. The eggshell ratio slightly increased with the addition of 60 mg Mn/kg, however, a significant decrease was observed with the addition of 120 mg Mn/kg (8.217 %) in comparison to the control (8.348 %). Conversely, an increase in the supplemental Mn level in the diet resulted in a corresponding increase in the value of eggshell-breaking strength. The highest eggshell-breaking strength was achieved with 120 mg Mn/kg (1.522 kg) addition. Likewise, despite the lack of statistical significance between the treatments, the maximum egg surface area was attained through 20 mg Mn/kg (1.522 kg) supplementation.

B addition to the breeder quail diet did not yield a statistically significant impact on either egg-specific gravity or egg surface area. However, including B in the diet had a significant impact ( $P \leq 0.01$ ) on the eggshell ratio and the shell-breaking strength values. The highest and lowest eggshell ratio values were achieved with 160 mg B/kg (8.409 %) and 40 mg B/kg supplementation (8.197 %), respectively. The eggshell-breaking strength values were highest when supplemented with 20 and 160 mg B/kg, resulting in 1.560 and 1.550 kg, respectively. Conversely, the lowest value was observed in the control group and with 80 mg B/kg supplementation, resulting in 1.455 and 1.405 kg, respectively. Statistical significance was observed in the difference between the treatments giving the highest and lowest values for eggshell ratio and eggshell breaking strength. However, the eggshell ratio values did

not exhibit any statistically significant difference between the control group and the B treatments.

The impact of Mn and B co-addition at varying doses (Mn  $\times$  B interactions) to breeder quail ration on egg-specific gravity and egg surface area values was also found to be insignificant. The study revealed that the eggshell ratio and eggshell breaking strength values exhibited a statistically significant increase only in the 60  $\times$  0 (60 mg Mn/kg + 0 mg B/kg) and 120Mn  $\times$  20B interactions, as compared to the control group (0x0 interaction, 0 mg Mn/kg + 0 mg B/kg).

### 3.3. Bone characteristics

The impact of various levels of Mn and B supplementation in the rations on the biomechanical properties of the tibia in breeding quail is given in Table 5. The addition of Mn did not have a significant impact on bone thickness, bone wall thickness, bone cross-sectional area, and shear fracture energy values when compared to the control group. The study revealed that there was no significant variation in the bone cross-sectional area values between the control (3.582 mm<sup>2</sup>) and 120 mg Mn/kg (3.632 mm<sup>2</sup>) groups. However, a statistically significant difference was observed between 60 (3.471 mm<sup>2</sup>) and 120 mg Mn/kg treatments. Furthermore, both doses of Mn increased ultimate shear force and shear stress values compared to the control. The maximum values of shear force and shear stress were attained at 137.147 N and 39.525 N/mm<sup>2</sup>, respectively, with 60 mg Mn/kg addition.

The addition of dietary B had no statistically significant effect on bone thickness. Furthermore, with the exception of the 40 mg B/kg dosage, the use of supplementary B did not yield a statistically significant impact on the values of bone wall thickness and bone cross-sectional area in comparison to the control. The addition of B between 20 and 80 mg/kg resulted in a significant and positive impact on both ultimate shear force and shear stress parameters compared to the control. Conversely, the highest dose of B (160 mg/kg) had an adverse effect



**Table 4**

The effect of different levels of manganese and boron supplementation to breeder quail rations on eggshell quality.

Addition of Mn and B levels (mg/kg)	Egg specific gravity (g/cm <sup>3</sup> )	Egg surface area (cm <sup>2</sup> )	Eggshell ratio (%)	Eggshell breaking strength (kg)
<b>Mn level (mg/kg)</b>				
0	1.07ab	24.17	8.34a	1.46b
60	1.07a	24.00	8.41a	1.49ab
120	1.07b	24.41	8.21b	1.52a
SEM	0.0005	0.14	0.029	0.016
P-value	0.04	0.137	0.0001	0.04
<b>B level (mg/kg)</b>				
0	1.07	23.99	8.36ab	1.45 cd
20	1.07	24.47	8.37ab	1.56a
40	1.07	24.25	8.19b	1.49bc
80	1.07	24.17	8.28bc	1.40d
160	1.07	24.09	8.40a	1.55ab
SEM	0.0007	0.18	0.03	0.020
P-value	0.18	0.43	0.002	<0.0001
<b>MnxB interaction (mg/kg)</b>				
0x0	1.07	24.01	8.45bc	1.47bcdef
0x20	1.07	24.24	8.52ab	1.48bcdef
0x40	1.06	24.14	8.19de	1.46cdef
0x80	1.07	24.33	8.08efg	1.36 g
0x160	1.07	24.14	8.49ab	1.54bc
60x0	1.07	24.29	8.66a	1.49bcde
60x20	1.07	24.15	8.18def	1.52bcd
60x40	1.07	24.30	8.41bc	1.45cdefg
60x80	1.07	23.73	8.27 cd	1.41efg
60x160	1.07	23.55	8.56ab	1.57ab
120x0	1.06	23.67	7.99 fg	1.39 fg
120x20	1.07	25.02	8.42bc	1.66a
120x40	1.06	24.32	7.99 g	1.57ab
120x80	1.07	24.46	8.51ab	1.43defg
120x160	1.07	24.59	8.17defg	1.53bcd
SEM	0.001	0.31	0.06	0.03
P-value	0.10	0.25	<0.0001	0.019

on these parameters. The highest values for shear force and shear stress were obtained at 80 mg B/kg addition rate, resulting in 137.930 N and 38.490 N/mm<sup>2</sup>, respectively.

The results indicated that 20 mg B/kg addition did not have a significant effect on the shear fracture energy when compared to the control. However, supplementation with 40–80 mg B/kg resulted in a statistically significant increase in shear fracture energy. The addition of 160 mg B/kg led to a decrease in the shear fracture energy. The experimental results indicated that the addition of 80 and 160 mg/kg supplementary B resulted in the highest and lowest values of shear fracture energy, which were measured as 80.246 and 56,411, respectively.

The concurrent application of Mn and B did not yield a statistically significant impact on bone thickness compared to the control, nor did either element when added individually. However, the combined addition of Mn and B to the diets resulted in a noteworthy increase in bone wall thickness and bone cross-sectional area. 120x0 interaction (120 mg Mn/kg + 0 mg B/kg) resulted in the highest bone wall thickness (0.509 mm) and bone cross-sectional area (3.928 mm<sup>2</sup>). With the exception of the 0x160 (0 mg Mn/kg + 160 mg B/kg) interaction, the ultimate shear force and shear stress values exhibited a generally positive impact across all Mn and B interactions in comparison to the control (0x0 interaction). The 60x20 (60 mg Mn/kg + 20 mg B/kg) and 120x80 (120 mg Mn/kg + 80 mg B/kg) interactions exhibited the highest ultimate shear force and shear stress values, whereas the 0 × 160 (0 mg Mn/kg + 160 mg B/kg) interaction demonstrated the lowest values. The study revealed a significant disparity in the impact of the co-application of Mn and B to breeder quail diets at varying concentrations on shear fracture energy. The interaction of 60 × 160 and 120 × 20 resulted in a noteworthy reduction in shear fracture energy. Conversely, the combinations of 0 × 80 (0 mg Mn/kg + 80 mg B/kg), 60 × 20, 120 × 40 (120 mg Mn/kg +

40 mg B/kg), and 120 × 80 led to the statistically significant increase in shear fracture energy when compared to the control. The highest shear fracture energy value of 87.044 N mm was observed in the 120x40 interaction.

The parameters pertaining to the impact of the addition of Mn and B, either individually or in combination, on the mineral content of the femur bone are presented in Table 6. The mineral contents of femoral Mn, B, iron (Fe), calcium (Ca), phosphorus (P), and magnesium (Mg) in breeding quail were significantly impacted by the level of Mn added to their rations. The inclusion of Mn supplementation in the diet of breeding quail resulted in a significant increase in the bone Mn, Fe, P, and Mg levels. However, there was no significant impact on the Ca content. Moreover, the introduction of supplementary Mn resulted in a noteworthy reduction in bone B concentration in comparison to the control group. The study revealed that the highest concentrations of Mn and Fe were detected in the sample treated with 120 mg/kg Mn. Conversely, the sample treated with 60 mg/kg B exhibited the highest levels of Ca, P, and Mg.

All levels of B supplementation and Mn × B interactions had a significant impact on the entire femoral minerals examined. The addition of B to the breeder quail feed resulted in a significant increase in the contents of Mn, B, and Fe in the bones, as compared to the control. A statistically significant gradual increase in bone B content was observed as the level of B added to the quail diet was increased. The statistical significance of the increase in bone Mn content was observed solely with the addition of 40 mg/kg B, resulting in a value of 17.739 mg/kg. On the other hand, a statistically significant increase in Fe content (102.488 mg/kg) was observed in the 160 mg B/kg application. On the contrary, the addition of over 20 mg/kg B to the diet of quails led to a significant decrease in the Ca, P, and Mg levels in the bones of quails as a whole.

A statistically significant increase ( $P \leq 0.01$ ) in the concentration of Mn in bone tissue was noted as the Mn content increased in Mn × B interactions. The peak Mn concentration in bone was achieved at the 120x40 interaction (120 mg Mn/kg + 40 mg B/kg), resulting in a value of 24.332 mg Mn/kg. A significant reduction in the bone Mn content was observed upon increasing the B level to 80 and 120 mg/kg in the interaction. The study revealed that the 120x40 interaction resulted in the highest bone Mn content of 24.332 mg/kg. Whereas, the femoral Mn content in 120x80 and 120 × 160 (120 mg Mn/kg + 160 mg B/kg) interactions were 19.917 and 17.462 mg/kg, respectively. Similarly, an increase in B content in Mn × B interactions led to a significant increase in bone B content ( $P \leq 0.01$ ). The highest bone B content was obtained in the 0 × 160 interaction (0 mg Mn/kg + 160 mg B/kg). However, as the level of Mn in the interaction was increased, a reduction in bone B concentration was observed. The femoral B content was 67.774 mg/kg in the 0 × 160 interaction (0 mg Mn/kg + 160 mg B/kg), whereas the bone B content in the 60 × 160 and 120x160B interactions were 54.115 and 56.642 mg/kg, respectively.

Mn × B interactions other than 0x80 (0 mg Mn/kg + 80 mg B/kg) and 60 × 20 (60 mg Mn/kg + 20 mg B/kg) resulted in a significant increase in femur bone Fe content in quails ( $P \leq 0.01$ ). Femoral Fe content was determined as 86.220 mg/kg in the control group, while the highest Fe content was observed as 124.93 mg/kg in the 120 × 160 interaction.

The study revealed that there was no discernible pattern in the impact of Mn and B interactions on the levels of Ca and P in bones. The bone Ca content attained its maximum value with 0 × 20 interaction (181838.120 mg Ca/kg), exhibiting a statistically significant difference ( $p \leq 0.01$ ) when compared to the control (176227.910 mg Ca/kg). Similarly, the 0 × 20 (132218.400 mg P/kg), 60 × 80 (131199.850 mg P/kg), and 120x20 (130601.360 mg P/kg) interactions resulted in the highest bone P contents, despite being categorized under the same group as the control (130162.100 mg P/kg). Other Mn and B interactions led to a statistically significant decrease in Ca and P content in bone compared to the control.

The 0 × 40, 0 × 80, and 0 × 160 interactions resulted in a notable

**Table 5**

The effect of different levels of manganese and boron supplementation to rations on the tibia biomechanical properties of breeding quail.

Addition of Mn and B levels (mg/kg)	Bone thickness (mm)	Bone wall thickness (mm)	Bone cross-sectional area (mm <sup>2</sup> )	Ultimate shear force (N)	Shear stress (N/ mm <sup>2</sup> )	Shear fracture energy (N.mm)
<b>Mn level (mg/kg)</b>						
0	2.94	0.45	3.58ab	125.70c	35.32c	68.61
60	2.90	0.45	3.47b	137.17a	39.52a	66.00
120	2.95	0.46	3.63a	133.88b	37.08b	68.21
SEM	0.02	0.00	0.04	0.99	0.437	1.66
P-value	0.26	0.09	0.028	<0.0001	<0.0001	0.49
<b>B level (mg/kg)</b>						
0	2.92	0.45a	3.56a	130.08b	36.71b	62.87b
20	2.93	0.46a	3.65a	137.05a	37.65ab	63.66b
40	2.90	0.43b	3.37b	130.45b	38.67a	74.87a
80	2.94	0.45a	3.58a	137.93a	38.49a	80.24a
160	2.95	0.46a	3.63a	125.71c	35.01c	56.41c
SEM	0.03	0.004	0.05	1.28	0.564	2.152
P-value	0.76	0.001	0.007	<0.0001	0.0004	<0.0001
<b>MnxB interaction (mg/kg)</b>						
0x0	2.94	0.42ef	3.37ef	117.39f	34.76 g	67.95def
0x20	2.92	0.44d	3.51cdef	136.53bcd	38.88bcd	64.17def
0x40	2.86	0.45d	3.41def	139.69bc	40.88abc	64.86def
0x80	2.97	0.47bc	3.73abc	133.76cde	35.87efg	82.81abc
0x160	3.03	0.48b	3.87ab	101.14 g	26.21i	63.28ef
60x0	2.90	0.43de	3.37ef	140.21b	41.59ab	60.18f
60x20	2.90	0.48b	3.66bcd	155.18a	42.43a	79.08abc
60x40	2.88	0.45d	3.45def	121.11f	35.09 fg	72.70cde
60x80	2.90	0.44de	3.42def	129.51e	37.82def	74.29bcd
60x160	2.90	0.44de	3.43def	139.71bc	40.68abc	43.77 g
120x0	2.94	0.50a	3.92a	132.64de	33.79gh	60.49f
120x20	2.97	0.47b	3.78abc	119.44f	31.65 h	47.75 g
120x40	2.95	0.41f	3.25f	130.55de	40.05abcd	87.04a
120x80	2.95	0.45d	3.60cde	150.51a	41.77a	83.62ab
120x160	2.94	0.45 cd	3.58cde	136.28bcd	38.13cde	62.17ef
SEM	0.05	0.008	0.09	2.21	0.976	3.72
P-value	0.91	<0.0001	0.0006	<0.0001	<0.0001	<0.0001

**Table 6**

The effect of different levels of manganese and boron supplementation to breeding quail ration on femoral mineral content.

Addition of Mn and B levels (mg/kg)	Mn (mg/kg)	B (mg/kg)	Fe (mg/kg)	Ca (mg/kg)	P (mg/kg)	Mg (mg/kg)
<b>Mn level (mg/kg)</b>						
0	11.1c	40.127a	89.6b	172017.5	127742.1b	7724.7b
60	16.7b	35.846b	97.2a	174145.7	129313.3a	8093.5a
120	20.1a	37.318b	100.6a	172901.9	128023.7b	7980.0a
SEM	0.44	0.64	1.95	816.5	371.178	47.3
P-value	<0.0001	0.0003	0.0028	0.20	0.01	<0.0001
<b>B level (mg/kg)</b>						
0	15.2b	23.333e	94.9b	175170.5a	129789.9ab	8055.9a
20	16.0ab	29.037d	95.6ab	175499.3a	130688.5a	8025.8ab
40	17.7a	33.493c	100.5ab	168905.6b	126375.1c	7760.1c
80	15.7b	43.445b	85.6c	175214.8a	128636.5b	7871.9bc
160	15.2b	59.510a	102.4a	170318.4b	126308.4c	7950.1ab
SEM	0.57	0.82	2.52	1053.67	477.27	61.04
P-value	0.0394	<0.0001	0.0018	0.0004	<0.0001	0.0152
<b>MnxB interaction (mg/kg)</b>						
0x0	11.2f	26.3 g	86.2ef	176227.9bc	130162.1abc	8058.7ab
0x20	11.3f	27.7 fg	97.5cde	181838.1a	132218.4a	8033.7abc
0x40	9.7f	34.8e	89.2ef	159658.0f	121527.2 g	7304.4e
0x80	11.7f	43.9 cd	78.9f	172684.5cde	126191.1ef	7500.7de
0x160	11.5f	67.7a	96.1cde	169679.2de	128611.9bcde	7726.0 cd
60x0	16.2de	18.6 h	105.3bc	173099.9cde	130457.9abc	8037.2ab
60x20	16.0de	28.1 fg	85.1ef	169690.5de	129245.8bcd	8097.8ab
60x40	19.1bcd	32.1e	117.8ab	174815.6 cd	130382.2abc	8013.0ab
60x80	15.4e	46.1c	91.4de	180495.6ab	131199.8ab	8213.7a
60x160	16.8de	54.1b	86.4ef	172626.8cde	125280.7f	8105.6ab
120x0	18.2bcd	24.9 g	93.1de	176183.6bc	128749.8 cd	8071.6ab
120x20	20.8b	31.2ef	104.2 cd	174969.1c	130601.3abc	7945.8abc
120x40	24.3a	33.5e	94.3cde	172243.3cde	127215.9def	7962.7abc
120x80	19.9bc	40.1d	86.5ef	172464.2cde	128518.6cde	7901.4bc
120x160	17.4cde	56.6b	124.9a	168649.3e	125032.7f	8018.6ab
SEM	1.33	1.42	4.361	2439.800	825.887	105.448
P-value	0.0099	<0.0001	<0.0001	<0.0001	<0.0001	0.0112

reduction in the Mg content of quail bone compared to the control group ( $0 \times 0$ ). Except for the three interactions, the bone Mn content was not significantly affected by other Mn  $\times$  B interactions. The  $60 \times 80$  interaction exhibited the greatest concentration of Mg in bone tissue, with a value of 8213.772 mg Mg/kg.

#### 4. Discussion

Manganese (Mn) plays a crucial role in the metabolic pathways of carbohydrates, amino acids, and lipids. Therefore, Mn has an important function in supporting growth and performance, facilitating matrix formation and bone development, and promoting the optimum eggshell formation and quality (Palacios, 2006; Kutlu, 2014; Dermience et al., 2015; Olgun, 2017; Arıcı et al., 2019). The current study has demonstrated that the only breeder quail performance parameter that displayed a noteworthy improvement following Mn supplementation was the average body weight gain. However, as per previous studies, there was a significant disparity in the impact of Mn depending on the amount used (Olgun, 2017). The addition of 60 mg/kg of Mn to the ration led to a significant decrease in the egg weight as compared to the control group. But 120 mg Mn/kg addition to the diet of quail resulted in an increase in the egg weight. In addition, the addition of 60 mg Mn/kg did not demonstrate a statistically significant effect on the evaluated eggshell quality parameters in the conducted experiments, when compared to the control group. The incorporation of 120 mg Mn/kg resulted in a significant reduction in eggshell rate, accompanied by an increase in eggshell breaking strength. The application of both Mn doses resulted in an increase in the ultimate shear force and shear stress values in comparison to the control. The highest ultimate shear force and shear stress values were obtained by the addition of 60 mg/kg Mn to the ration. However, the cross-sectional area of the bone exhibited a significant increase in 120 mg/kg supplementary Mn compared to 60 mg/kg of Mn application. The addition of supplementary Mn resulted in a significant increase of bone Mn, Fe, P, and Mg levels, whereas a substantial reduction in B levels was observed in comparison to the control. Based on the analysis of bone mineral content, the inclusion of 60 mg/kg of Mn would be adequate. The findings indicate that Mn is a crucial micronutrient that contributes to the enhancement of performance capabilities, eggshell, and bone features in Japanese quails, as reported in previous studies (NRC, 1994; Çimrin and Tunca, 2012; Olgun, 2017; Arıcı et al., 2019).

A positive correlation was detected between the addition of B to the diet and the amount of B in the femoral tissue of breeder quails. This result was also emphasized by Wilson and Ruzsler (1998). However, among the analyzed performance indicators, only the average weight gain was significantly affected by the addition of B to the rations. The 20 mg/kg supplementary B resulted in the highest increase in body weight. The addition of B to the diet caused a slight increase also in egg production, egg weight, and egg mass values compared to the control. Simultaneously, decreases in both feed intake and feed conversion ratio values were detected. It was concluded that the addition of B to the diet had a positive effect on growth performance, as previously documented by Wilson and Ruzsler (1998); Kurtoğlu et al. (2001); Bozkurt et al. (2007). Results on observed performance characteristics indicate that dietary supplementation of 20 mg/kg B is adequate for Japanese quail production. In a similar vein, Özdemir et al. (2016) revealed that the inclusion of 25 mg/kg B resulted in a significant increase in the body weight of quails. The highest values for eggshell characteristics were also exhibited with 20 mg/kg B supplementation. Therefore, the addition of 20 mg/kg B to the rations is sufficient for optimum performance and eggshell quality. Exceeding this amount of B may have adverse effects on both the performance and eggshell quality of Japanese quails (Kara, 2022).

The supplementation of Mn or B did not significantly impact bone thickness, bone wall thickness, and bone cross-sectional area. However shear force and shear stress values were positively affected by both Mn

and B additions. B supplementation had a positive impact on shear fracture energy. The optimal values for the aforementioned parameters were achieved through the application of 60 mg/kg Mn and 80 mg/kg B in individual treatments. However, based on the available data on the biomechanical properties of the tibia, supplementation of 20–40 g/kg B would be adequate. Prior research has suggested that B plays a significant role in the bone development, proliferation, and mineralization of animals (Wilson and Ruzsler, 1998; Uysal et al., 2009; Olgun et al., 2012; Abdelnour et al., 2018). Therefore, the addition of B in the diet of laying hens resulted in an increase in shear force, shear stress, and shear fracture energy values, as well as a decrease in joint disorders (Mizrak et al., 2008; Koçbeker et al., 2017).

The levels of B supplements had a significant impact on the stored minerals in bones. The increase in dietary B was positively correlated with an increase in femoral B content. The addition of B to the quail feed for breeding resulted in a significant increase in the contents of Mn and Fe in the bones as compared to the control group. The addition of boron at a rate of 20 mg/kg resulted in a slight increase also in the Ca and P contents of bones. Nevertheless, the addition of over 20 mg/kg of B in the quail diet frequently resulted in a significant decrease in the Ca, P, and Mg levels in the bones. As previously indicated, B has been documented to have advantageous impacts on poultry skeletal health, mineral homeostasis, and the quality of eggshells. Boron is regarded as a plausible vital nutrient for the metabolism and utilization of diverse micronutrients that could potentially impact the bone mineral content in poultry (Olgun and Yildiz, 2014; Olgun and Bahtiyarca, 2015; Bozkurt and Küçükylmaz, 2015; Abdelnour et al., 2018). Concurrent with our findings, contemporary research has demonstrated the efficacy of B in the metabolic processes of essential minerals such as Ca, P, Mg, and Cu, as well as vitamin D, which have a significant impact on bone structure and overall health of poultry (Hunt and Nielsen, 1986; Nielsen, 1990; Wilson and Ruzsler, 1998; Kurtoğlu et al., 2007; Karabulut and Eren, 2006; Olgun et al., 2012; Kaya and Macit, 2018; Kaya, 2020). Consistent with prior research, the findings indicated that the addition of B to ration also confers a beneficial impact on bone growth and mineral metabolism in Japanese quail.

The interaction of Mn and B had a significant impact on the average live weight gain and egg weight values, which were both considered performance criteria. The B and Mn are recognized as crucial nutrients for the optimal metabolism of Ca, which is essential for maintaining bone health and eggshell quality, as well as sustaining poultry performance (Nielsen et al., 1998; Elliot and Edwards, 1992; Hunt and Idso, 1999; Olgun, 2017; Spears, 2018; Wang et al., 2022).

A proportional increase in the Mn concentration in bone was observed with increasing Mn in Mn  $\times$  B interactions. The highest bone Mn content was recorded in the interaction of  $120 \times 40$ . Likewise, a positive correlation was observed between the amount of B involved in the interaction and the bone B concentration relative to the control. The observed interaction indicated a reduction in the bone B content upon the introduction of Mn. The highest bone B content was obtained in the  $0 \times 160$  interaction. However, bone Mn concentration showed an increasing trend until B concentration reached 40 mg/kg in the Mn  $\times$  B interactions. The  $120 \times 40$  interaction demonstrated the highest bone Mn concentration.

In general, a majority of the interactions between Mn and B resulted in an elevation of Fe content in the femur bone. The highest concentration of Fe in the bone was obtained in the  $120 \times 160$  interaction. According to Pradhan et al. (2018), B actively participates in the mineral metabolism of animals and interacts with various nutrients such as Ca, Mg, Mn, Mo, K, Cu, and vitamin D3. However, Bai et al. (2014) reported that the inclusion of Mn in the diet decreased the Fe content in the liver and duodenum of hens. Moreover, high doses of Mn can impede Fe absorption in young chickens (Baker and Halpin, 1991). Therefore, the deposition of Fe in bones is primarily related to the addition of B into the diet. Similarly, Long et al. (2023) reported that the accumulation of Fe in plants is also positively correlated with the B concentration. The study

did not identify a discernible pattern in the impact of the Mn and B interaction on Ca, P, and Mg contents in bone tissue. However, the 0x20 interaction yielded significantly higher Ca and P in the bone compared to the control (0x0). Abdelnour et al. (2018) have proposed that the consumption of B may enhance the absorption of Ca, P, and Mg, resulting in a reduction in the excretion of these minerals. Thus, the increase in Ca, P, and minor Mg levels within bones is predominantly a consequence of B's involvement in the interaction. Previous research has also confirmed B's potential as a crucial nutrient that could impact the metabolism and utilization of diverse micronutrients in poultry, consequently affecting bone mineral content (Abdelnour et al., 2018; Bozkurt and Küçükylmaz, 2015; Olgun and Bahtiyar, 2015; Olgun and Yıldız, 2014).

## 5. Conclusions

The research findings collectively indicate that the optimal production of Japanese quail can be achieved through the use of a combination of 60–120 mg/kg Mn and 20 mg/kg B. The literature extensively covers the biological responses of poultry to the supplementation of Mn and B. However, there is significant variation in the existing literature concerning the impact of Mn and B supplementation on performance, eggshell quality, and bone biomechanical properties. Furthermore, a limited number of research studies have assessed the synergistic effects of incorporating both components as nutritional supplements. Hence, it is recommended that further comprehensive investigations be conducted to assess the combined effects of Mn and B as dietary supplements in the feeding regimen of Japanese quail.

## CRedit authorship contribution statement

**Muhammet Ali Kara:** Conceptualization, Data curation, Formal analysis, Funding acquisition, Investigation, Methodology, Project administration, Resources, Software, Supervision, Validation, Visualization, Writing – original draft, Writing – review & editing.

## Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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