



The effects of *Saccharomyces cerevisiae* on the morphological and biomechanical characteristics of the tibiotarsus in broiler chickens

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Abstract. The aim of this study is to examine the effects of different levels of the feed supplement *Saccharomyces cerevisiae*, a yeast metabolite, on broiler tibiotarsus traits and to reduce leg problems by identifying the pathological changes in leg skeletal system. Thus, reducing leg disorders due to the skeletal system, the cause of significant economic losses in our country (Turkey), was investigated by the supplementation of *Saccharomyces cerevisiae* in broiler feed.

In the study, 300 male day-old, Ross 308 broiler chicks were used. Experiment groups were designed as follows: control; 0.1 % *Saccharomyces cerevisiae*; 0.2 % *Saccharomyces cerevisiae*; 0.4 % *Saccharomyces cerevisiae*. The experimental diets were chemically analyzed according to the methods of the Association of Official Analytical Chemists. Twelve groups were obtained, including three replicates for each experimental group. Each replicated group was comprised of 25 chicks, and thus 75 chicks were placed in each experimental group. After 42 days, broiler chickens were slaughtered. Tibiotarsi were weighed with a digital scale, and the lengths were measured with a digital caliper after the drying process. Cortical areas were measured with the ImageJ Image Processing and Analysis Program. A UTEST Model-7014 tension and compression machine and a Maxtest software were used to determine the bone strength of the tibiotarsus. The severity of the tibial dyschondroplasia lesion was evaluated as 0, +1, +2 and +3. Crude ash, calcium and phosphorus analyses were performed to determine the inorganic matter of tibiotarsi. For radiographic evaluations of epiphyseal growth plates, tibiotarsi from the right legs were photographed in lateral and craniocaudal positions and examined. Statistical analyses were performed with the SPSS statistics program.

It was observed that the use of *Saccharomyces cerevisiae* as a feed supplement led to an increase in the bone traits of broiler chickens. Optimum results for bone mineral content, biomechanical traits and strength were provided by the addition of 0.2 % *Saccharomyces cerevisiae* in broiler feed.

As a result, the use of yeast as feed supplements in broilers is considered to be an economic and convenient way of providing animal welfare and preventing commercial losses due to leg problems.

1 Introduction

A common problem in the broiler chicken industry is leg problems. Excessive breast muscle accumulation combined with an inadequate skeletal development, gender, genetics, feeding, the composition of food, the incubation period, infectious diseases, environmental stress factors, maintenance and management factors affect the frequency and severity of leg problems (Bradshaw et al., 2002; Oviedo-Rondón et al., 2006a, b).

In genetically selected broiler chickens, the disproportionate increase in breast muscle (25–30 % of body weight) compared to other muscle leads to an unbalanced weight distribution on the femur and tibiotarsus physiologically (Havenstein et al., 2003, 2004, 2007; Oviedo-Rondón, 2007); depending on age, chickens become more passive (Kestin et al., 2001). These chickens also shorten the time they are active and consequently they place greater stress on the feet. Therefore, the femur, tibiotarsus and joints of these bones are exposed to more stress in wide breasted broilers than in the traditional line (Abourachid, 1993).

Leg abnormalities cause pain and discomfort for broilers (Danbury et al., 2000; McGeown et al., 1999), and severe lameness limits the broilers' access to feeders and water (Weeks et al., 2000; Knowles et al., 2008). Consequently, chickens can be exposed to hunger, thirst, and dehydration (Butterworth et al., 2002). In addition, weight loss and poor-quality products are an economic problem for the poultry industry (Yalcin et al., 1998; Kestin et al., 1999), and leg problems are a serious welfare problem for broiler chickens (European Commission, 2000).

Probiotics are generally used as an alternative additive for competition and elimination of bacterial pathogens in the poultry industry (Barrow, 1992). As a probiotic, *Saccharomyces cerevisiae* cell walls contains beta-glucans (Kollár et al., 1995, 1997), and beta-glucans in yeast have immunomodulating and growth-promoting effects (Park et al., 2001). In addition, Plavnik and Scott (1980) suggested that the positive effect of the yeast on the egg shell may have positive effects on the bone and may increase the bone strength. It was also reported that the addition of *Saccharomyces cerevisiae* (yeast) to the feed increases the production of calcitriol receptors (McDonnell et al., 1989).

Calcitriol increases blood calcium (Ca) levels by promoting the absorption of dietary Ca from the gastrointestinal tract and increases renal tubular reabsorption of Ca, thus reducing the loss of Ca in the urine. Calcitriol also stimulates the release of Ca from bone. The observation that calcitriol stimulates the release of Ca from bone seems contradictory, given that sufficient levels of serum calcitriol generally prevent overall loss of Ca from bone. It is believed that the increased levels of serum Ca resulting from calcitriol-stimulated intestinal uptake causes bone to take up more Ca than it loses by hormonal stimulation of osteoclasts (Voet and Voet, 2004).

The aim of this study is to investigate the effects of different levels of *Saccharomyces cerevisiae* on the tibiotarsus in broiler chickens. In addition, by reducing the pathologic changes, we have investigated reducing leg disorders caused by the skeletal system; they are the reason for significant economic loss and threaten broiler welfare.

2 Material and methods

2.1 Animals, groups and feeding

The experiment was carried out on broiler chicks ($n = 300$) purchased from CP Inc. Com, Bursa, Turkey. The experimental protocols were approved by the Animal Care and Use Committee of Uludag University (Turkey) and are in accordance with the National Institute of Health Guide for the Care and Use of Laboratory Animals. The study was carried out with the permission of Uludag University (Turkey) Animal Experimentation Local Ethics Committee (Approval No. 2013-02/06).

Three hundred 1-day-old male chicks were randomly selected and distributed into four groups with three replicates of 25-day-old chicks in each. Thus, 75 chicks were placed in each experimental group. A basal diet was supplemented with *Saccharomyces cerevisiae* live yeast culture (Yea-Sacc 1026: 1×10^9 CFU g⁻¹, Alltech, Nicholasville). All diets were formulated to provide 3100 kcal of ME kg⁻¹ and to meet the amino acid ratios and all other nutrients as suggested by NRC (1994). The compositions of the diets are presented in Table 1. Depending on the experimental design, groups are (i) control (C), (ii) 0.1 % *Saccharomyces cerevisiae* added diet (Y1), (iii) 0.2 % *Saccharomyces cerevisiae* added diet (Y2), (iv) 0.4 % *Saccharomyces cerevisiae* added diet (Y4).

The experimental diets were chemically analyzed according to the methods of the Association of Official Analytical Chemists (AOAC, 2000). The metabolizable energy (ME) levels of the diets were estimated using the equation of Carpenter and Clegg (Leeson and Summers, 2001): $ME \text{ (kcal kg}^{-1}\text{)} = 53 + 38 [(CP, \%) + (2.25 \times \text{ether extract, } \%) + (1.1 \times \text{starch, } \%) + (1.05 \times \text{sugar, } \%)]$. The dry matter (DM) and ash content were determined by drying the feed to a constant weight at 103 °C and combustion at 550 °C, respectively. The diethyl ether extract was analyzed with the Soxhlet method (ISO 1444, 1973). Crude fiber was determined using the Association of Official Analytical Chemists methods (method 962.09 and 985.29; AOAC, 1995). The Kjeldahl method (ISO 5983-1, 2005) was used to determine CP ($6.25 \times N$).

The chicks were housed in an environmentally controlled poultry house with the floor covered with wood shavings and kept dry throughout the study. Feed and water were provided ad libitum. Feeding lasted 42 days. The animals were fed a commercial broiler starter diet (CP Inc. Com, Bursa, Turkey) for the first 20 days, a pelleted grower diet (CP Inc. Com,

Table 1. The nutrient composition of the experimental diets.

Nutrient content	Starter (0–20 days)	Grower (21–35 days)	Finisher (36–42 days)
Metabolic energy, kcal kg ⁻¹	3008	3068	3164
Crude protein, %	22.50	21.50	20.00
Crude fibre, %	3.40	3.00	3.00
Oil, %	6.00	5.20	5.00
Ash, %	5.00	6.00	6.00
Lysine, %	1.40	1.30	1.20
Methionine, %	0.60	0.50	0.44
Calcium, %	0.80	0.75	0.70
Vitamin mineral premix*	27.40	27.40	26.70

* Provided the following per kilogram of diet: vitamin A 10 000 IU, vitamin D3 5000 IU, vitamin D3 4000 IU (only for finisher diet), vitamin E 75 mg, vitamin E 75 (50 mg only for finisher diet), phosphorus 7000 mg, sodium 2000 mg, manganese 120 mg, zinc 100 mg, selenium 0.30 mg, iron 40 mg, iodine 1.25 mg, copper 16 mg.

Bursa, Turkey) from 21 to 35 days of age and a finisher diet (CP Inc. Com, Bursa, Turkey) from 36 to 42 days of age. Ingredient and nutrient compositions of diets are shown in Table 1.

2.2 Measurements

Broiler chickens were slaughtered at the age of 42 days. The left and right legs were separated from the body at the level of *junctura coxae*. Soft tissues around the bones were dissected and removed to obtain the tibiotarsus. After the removal of the femur and tarsometatarsus, the remaining tibiotarsi were maintained at -20°C for further studies (Crenshaw, 1986). The tibiotarsi taken from the right leg were used for the evaluation of bone weight, length, cortical area and strength, and half of the tibiotarsi taken from the left leg (150 pieces) were used for the evaluation of tibial dyschondroplasia (TD) and epiphyseal growth plates, and the rest of the tibiotarsi from the left leg were used for the determination of bone ash, bone Ca and P levels.

2.2.1 Bone weight, length, cortical area and strength tests

The right tibiotarsi were left at room temperature to defrost, and then the bones were stored at room temperature for 2 weeks until they were dry. After the drying process, the bones were weighed with Precisa XB4200C digital scales (Precisa Instruments Ltd., Switzerland) and the lengths were measured with a Mitutoyo CDN-20C digital caliper (Mitutoyo Corp., Kawasaki, Japan).

For strength evaluation, diaphyseal sections were taken from each tibiotarsus with a thickness of 1 cm (Yildiz et al., 2009). Bone sections were numbered and photographed with the help of a Canon EOS 600D camera (Canon Inc., Japan). Photographs were transferred to a computer, and cortical areas of the diaphysis were measured with the ImageJ Im-

age Processing and Analysis Program (National Institutes of Health, Bethesda, Maryland, USA) (Doube et al., 2010). To determine the maximum strength of the tibiotarsus, a UTEST Model-7014 tension and compression machine (Utest Inc., Ankara, Turkey) with a 50 kN load cell were used with the aid of the Maxtest software. The section of diaphysis was placed between the jaws of the tension and compression machine. The section was subjected to a force at speed of 10 mm min^{-1} in the vertical direction until it was broken. When the section was broken, the process was stopped and the maximum breaking force (kilonewton, kN) was recorded (Yildiz et al., 2009).

2.2.2 Bone ash, calcium and phosphorus

Tibiotarsi stored at -20°C were removed and allowed to thaw at room temperature. After the bones were thoroughly cleaned from soft tissue, they were divided into halves and placed in jars containing ether for 4 days to remove the oil. Following this, the bones were kept at 105°C for 12 h in an incubator to be dried. Crude ash analyses were performed according to the method reported by AOAC (1960); Ca analyses were carried out using the spectrophotometric method and phosphorus (P) analyses by using the method of Gericke and Kurmies (1952).

2.2.3 Tibial dyschondroplasia

For the detection of TD, longitudinal sections were performed on the proximal epiphysis and metaphysis of tibiotarsi, and the epiphyseal cartilage and metaphysis were macroscopically examined. The severity of the TD lesion was evaluated as 0, +1, +2 and +3 according to Edwards and Veltmann (1983). Sections were kept in 10% formaldehyde for histopathological examination of the lesions. After fixation, specimens were decalcified in sodium-citrate-buffered formic acid and then embedded in paraffin blocks.

Table 2. Effects of *Saccharomyces cerevisiae* on broiler tibiotarsus weight, length, cortical area and strength.

Groups	Bone weight (g)	Bone length (mm)	Cortical area (cm ²)	Strength (kN)
C	10.45 ± 0.19 ^a	103.77 ± 0.50 ^a	0.34 ± 0.01 ^a	1.91 ± 0.11 ^a
Y1	10.51 ± 0.25 ^a	104.05 ± 0.69 ^a	0.34 ± 0.01 ^a	1.55 ± 0.07 ^b
Y2	11.14 ± 0.13 ^b	106.35 ± 0.39 ^b	0.38 ± 0.01 ^b	1.89 ± 0.09 ^a
Y4	11.14 ± 0.13 ^b	106.03 ± 0.52 ^b	0.34 ± 0.01 ^a	1.40 ± 0.07 ^b

Different superscripts indicate statistical differences ($P < 0.05$).

Sections 5–6 µm thick were taken from the prepared paraffin blocks. Sections were stained with haematoxylin–eosin (Dinev, 2009) and Mallory's triple staining (Culling et al., 1985) and examined under a light microscope.

2.2.4 Epiphyseal growth plates

Before performing strength tests, tibiotarsi from the right legs were photographed in lateral and craniocaudal positions and transferred to digital cassettes (Philips, Duodiagnost, the Netherlands) for radiographic evaluations. Cassettes were monitored using a computerized X-ray reading device (FCR CAPSULA XLII, Fujifilm, Japan), and then epiphyseal growth plates (Breugelmans et al., 2007) and deviations were evaluated.

2.3 Statistical analysis

Statistical analyses were performed with IBM SPSS (SPSS, Version 20.0; Chicago, IL). Data were tested for normal distribution and variance homogeneity assumptions. All the values were grouped and the means and standard errors were calculated. Data are stated as mean ± SE (standard error) of the mean (SEM). A chi-square test was used to evaluate the statistical differences in the degree of closure of epiphyseal growth plates and TD lesion scores among the groups (Veltmann and Jensen, 1980). For the statistical evaluation of other parameters, an analysis of variance (ANOVA) test was used. Differences were accepted as significant if $P < 0.05$. When the differences between the groups were significant ($P < 0.05$), differences were assessed by the Tukey test (Dowdy and Wearden, 1981).

3 Results

3.1 Bone weight, length and cortical area

There was no statistically significant difference between the C and Y1 groups and the Y2 and Y4 groups in terms of bone weight ($P > 0.05$). However, in the Y2 and Y4 groups, bone weight was found to be significantly increased compared to the C group ($P < 0.05$). There was no statistically significant difference between the C and Y1 groups in terms of bone length and cortical area ($P > 0.05$). However, there was a

Table 3. Effects of *Saccharomyces cerevisiae* on broiler tibiotarsus ash, calcium and phosphorus levels.

Groups	Ash (%)	Calcium (%)	Phosphorus (%)
C	43.88 ± 0.14 ^a	6.36 ± 0.17 ^a	3.54 ± 0.02 ^a
Y1	47.23 ± 0.26 ^{b,c}	6.44 ± 0.12 ^a	3.18 ± 0.01 ^b
Y2	45.52 ± 0.18 ^c	6.90 ± 0.17 ^a	3.73 ± 0.03 ^c
Y4	45.97 ± 0.20 ^c	7.90 ± 0.22 ^b	3.09 ± 0.01 ^d

Different superscripts indicate statistical differences ($P < 0.05$).

significant increase in bone length and cortical area in the Y2 group compared to the C group ($P < 0.05$) (Table 2).

3.2 Bone strength

There was no statistically significant difference between the C and Y2 groups and the Y1 and Y4 groups in terms of bone strength ($P > 0.05$). Although the Y1 and Y4 groups showed a significant decrease, the C and Y2 groups showed a significant increase in fracture strength compared with the Y1 and Y4 groups ($P < 0.05$) (Table 2).

3.3 Bone ash, Ca and P

The amount of bone ash was significantly increased in all the experimental groups (Y1, Y2, Y4) compared to the C group ($P < 0.05$). There was a significant increase in the amount of bone Ca in the Y4 group compared to the other groups ($P < 0.05$), but there was no significant difference between the C group and the Y1 and Y2 groups ($P > 0.05$). The amount of bone P was significantly decreased in Y1 and Y4 groups compared to the C group ($P < 0.05$), and in the Y2 group, there was found to be a significant increase compared to the C group ($P < 0.05$) (Table 3).

3.4 Tibial dyschondroplasia

At the end of the 42-day breeding period, broiler chickens were found to be affected by TD by an average of 79.3%. When the severity of TD cases (0, +1, +2, +3) were examined, it was observed that the TD severity decreased with increasing concentration of added yeast, but no statistically difference was observed between the groups in terms of TD incidence ($P > 0.05$) (Table 4).

3.5 Epiphyseal growth plates

There was no statistical difference between the groups in lateral and caudal deviation ($P > 0.05$). However, epiphyseal plates were found to be closed significantly earlier in the Y2 group than in the C group ($P < 0.05$). There was no statistically significant difference in the other groups ($P > 0.05$) (Table 5).

Table 4. Effects of *Saccharomyces cerevisiae* on tibial dyschondroplasia in broiler chickens.

Groups	Healthy		Tibial dyschondroplasia		
	Number	%	Number	%	
C	6	17.1	29	82.9	$P = 0.09$
Y1	6	17.1	29	82.9	
Y2	7	20.0	28	80.0	
Y4	10	28.6	25	71.4	

The mean difference is significant at the 0.05 level.

4 Discussion

4.1 Bone weight, length and cortical area

In the present study, the optimum bone development was observed especially in the Y2 group ($P < 0.05$), and the increase in bone weight, length and cortical areas was thought to be the result of the growth-promoting effects of beta-glucans in the yeast content. Supporting these results, Guenther et al. (2003) reported that beta-glucans are able to suppress osteoclast development and may enhance the development of osteoblasts. As reported by Onwurah et al. (2013), the contribution of yeast to broiler performance is more effective in the initial phase. Therefore, in the initial phase it is thought that yeast could not contribute enough bone development in the low-dose yeast group (Y1). It is also observed that the high-dose yeast group (Y4) cannot affect bone length, as reported by Rath et al. (1999) in spite of increased bone mineralization; there is not enough collagen accumulation. In the present study cortical area significantly increased in the Y2 group, but there is no significant difference among the other groups. The increased cortical area in the Y2 group could be the result of greater bone weight, length and P deposition of the tibiotarsus.

4.2 Bone strength

Plavnik and Scott (1980) reported that a 2.5 and 5 % yeast addition to the diet had definite improvements regarding the leg weakness of broilers. Arican (2012) reported that yeast culture did not have a statistically significant effect on bone strength in rabbits, but the group with 2 g kg⁻¹ yeast added had a higher bone breaking strength value than the other groups. Similarly, in the present study, a higher breaking strength was observed in the group with 0.2 % yeast added (Y2), with control group similar to the other yeast groups. As reported by Newman and Leeson (1998), the force applied to the bones is distributed by the cortical region and the endosteum. Therefore, the results are thought to be due to the increased cortical area in the Y2 group.

Akhavan-Salamat et al. (2011) reported that the yeast addition to the diet increased bone Ca levels in broiler chickens, which increased bone strength. In the present study, the high dose of yeast (Y4) caused a decrease in bone strength while

Table 5. Effects of *Saccharomyces cerevisiae* on epiphyseal growth plate of tibiotarsus in broiler chickens.

Groups	Closed epiphyseal plate		Non-closed epiphyseal plate		<i>P</i>
	Number	%	Number	%	
Y1	14	60.9	9	39.1	$P = 0.552$
Y2	20	83.4	4	16.6	$P = 0.022^*$
Y4	13	54.2	11	45.8	$P = 0.891$

* The mean difference is significant at the 0.05 level.

increasing the amount of bone ash and minerals. It is based on the report by Rath et al. (1999) that the bone may become more fragile despite increased mineral content. It may be associated with an inadequate amount of collagen in the bone structure.

4.3 Bone ash, Ca and P

Although Ghasemi et al. (2006) and Yildiz et al. (2011) reported that there is no statistical difference between groups in the amount of tibiotarsus ash through addition of yeast to broiler chickens, Ghasemi et al. (2006) reported that broiler chicks fed with yeast-supplemented feed had a marginally higher tibiotarsus ash content compared to chicks fed without a yeast supplement. In the present study, it was determined that the amount of bone ash was significantly increased in all experimental groups (Y1, Y2 and Y4) when compared to the C group ($P < 0.05$).

Akhavan-Salamat et al. (2011) reported that the addition of yeast to the diet increased bone Ca levels in broiler chickens. Simons et al. (1990) reported that the yeast was able to produce the phytase enzyme and that this enzyme was necessary to obtain inorganic P from phytate. Phytase also releases Ca from the insoluble salts of phytic acid and potentially makes Ca available for absorption in birds (Qian et al., 1997). Ghasemi et al. (2006) also observed that a linear increase in yeast in the feed increased Ca retention. In the present study, there was a significant increase in bone Ca in the Y4 group compared to the other groups ($P < 0.05$), but no significant difference among Y1, Y2 and C groups. It could be the result of higher microbial phytase activity in the Y4 group against others groups.

Although Thayer and Jackson (1975) reported that the addition of live yeast culture to the diet of chickens in the developmental period increased P utilization, in the present study, it is determined that there was only a significant increase in bone P in the Y2 group ($P < 0.05$) and there is a significant decrease in the Y1 and Y4 groups ($P < 0.05$). As reported by Suzer et al. (2015), the 0.2% yeast supplemented group had significantly higher plasma P level compared to the control group in broiler chickens. Therefore, it is thought that an in-

creased P level in the blood due to the addition of 0.2 % yeast caused increased deposition of P in the tibiotarsus.

4.4 Tibial dyschondroplasia

Thorp and Maxwell (1993) reported that TD affects 1–40 % of broiler chickens in commercial establishments, and 20–60 % of these animals show subclinical lesions. Edwards (1989) reported that in experimental studies the incidence of TD could be up to 80–90 %. In the present study, 79.3 % of broiler chickens were observed to be affected by TD in postmortem evaluations made after 42 days of growing period.

It has been reported that calcitriol has the ability to upregulate its own receptor activity, which occurs at the mRNA level, and thus calcitriol can automatically induce its own receptor protein (Costa et al., 1985; Pike, 1991). It is also reported that yeast increases the production of calcitriol receptors (McDonnell et al., 1989). In the present study, it was determined that there was no significant difference between the groups in terms of the presence of the TD in the different doses, but, when the percentages of TD severity in the groups were examined numerically, it was observed that the TD severity decreased while the yeast concentration increased. Positive improvements in TD incidence may be associated with increased calcitriol receptor activity by yeast supplementation.

4.5 Epiphyseal growth plates

Onwurah et al. (2013) reported that 5 g kg⁻¹ yeast can be supplemented in feed in the starter phase but should not exceed 1 g kg⁻¹ in the finisher phase for an optimum feed conversion ratio and body weight gain. According to these reports, in the present study, it is thought that in the Y1 group, yeast was insufficient, and in the Y4 group, yeast is excessive for the longitudinal growth of the bone, and it was suppressed on the basis of inadequate feed intake and feed conversion ratio.

The epiphyseal growth plate is responsible for the longitudinal development of the bone, and it is the basis for bone development (Hunziker, 1994; Price et al., 1994). Kim et al. (2009) and Lee et al. (2011) reported that yeast hydrolysate increased the proximal epiphyseal length of bone, accelerated its longitudinal extension and stimulated growth hormone secretion in young rats. There is no study of the effect of the yeast on the epiphyseal growth plate of broiler chickens. In the present study, the closure rates of epiphyseal growth plates were statistically different between the C and Y2 groups ($P < 0.05$). According to this, in the Y2 group, there was a significantly higher epiphyseal plate closure rate than in the C group.

5 Conclusions

In conclusion, when *Saccharomyces cerevisiae* was used as a feed additive, yeast increased bone mineral content and thus improved the biomechanical properties and durability of the bone. In addition, optimum results were obtained from the group with 0.2 % yeast (Y2) for bone mineralization, bone weight, length and cortical area width, bone strength, and curing effects on TD and epiphyseal growth plates. Using 0.4 % yeast (Y4) has no positive effects on bone biomechanical properties and durability, so it would be better to avoid a high dose of yeast so as not to increase the cost. Accordingly, it has been concluded that as a feed additive, *Saccharomyces cerevisiae* has beneficial and economical effects in terms of animal welfare and can prevent losses due to foot problems in commercial enterprises.

Data availability. Data are available on request from the corresponding author.

Competing interests. The authors declare that they have no conflict of interest.

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