

Effects of removing vitamins and trace minerals from finisher diets on production parameters, tibia strength and ash content in chicken bones

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Vitamins and trace minerals are necessary nutrients for normal functioning of the organism. The experiment was set up with 1400 chicken, divided into 7 groups, which were fed diets with no vitamin additives or vitamin + trace minerals from day 30 to 42. These additives were excluded 12, 8 and 4 days before the end of the fattening process. The following production parameters were monitored: body weight, weight gain, feed intake, feed conversion and mortality. On that basis the production index was calculated. Slaughtering parameters, ash content and tibia strength in bone were monitored. It was assumed that sufficient quantities of trace minerals and fat-soluble vitamins would be stored in the body. Great amounts of water soluble vitamins cannot be generally accumulated, but chickens will continue to obtain them from natural feedstuffs, after exclusion of premixes. The results show that exclusion of vitamin-mineral additives had no negative effects on final body weight, but feed conversion rate deteriorated after these additives were excluded for 8 and 12 days. As for slaughtering parameters (except for abdominal fat) and tibia strength, there were no negative effects. Removing only vitamins had more negative effects on bone ash contents than it was observed for the simultaneous removal of vitamins and minerals.

KEY WORDS: chicken / mineral / nutrition / removal / tibia / vitamin

A chicken diet must consist of energy, proteins, i.e. amino acids, fatty acids, vitamins and minerals. Having in mind that broiler nutrition costs are high (55-70% of the total production costs), opportunities to reduce them are being investigated. In support of that, there are many researches aiming to remove some nutrients for a certain period of time

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from the diet with no bad impact on production parameters. Many authors [Patience and Gillis, 1995, Kim *et al.* 1996, 1997]. have conducted studies evaluating effects of 7-day removal of vitamins and trace minerals from broiler chicken diets, but also from pigs in their final stages of fattening. Removing vitamins and trace minerals from the final stages of pig fattening caused no negative effects on body weight, feed conversion efficiency, carcass and meat quality. However, the results [Spurlock *et al.* 1998] were completely varied. Removal of these additives 44 days before the end of pig fattening led to negative consequences for growth and feed consumption. Three experiments were conducted [Teeter and Deyhim, 1996] to determine the effects of vitamin removal from broiler chicken diets on production characteristics. Animals that were fed diets free of premixes did not have reduced body weight, feed conversion efficiency and white meat yield. Removing only vitamins from the diet in the period from 28-49 days of fattening reduced daily gain, feed efficiency and yield of white meat. Removal of trace minerals from the diet within the same period had no negative effects on monitored parameters. It was surprising that removal of vitamins and trace minerals have less negative effects on the monitored parameters than the removal of vitamins alone. When vitamins were removed from the diet, performance trait values deteriorated due to the effect of trace minerals. Moravej *et al.* [2012]. reported similar findings in another experiment, where an increased consumption of trace minerals led to reduced production characteristics. There were significant differences in body weight, bone characteristics and ash content when the vitamin premix had been removed after 36-42 days of life, but not during days 29-35. The combined removal of vitamins and trace minerals from the broiler diet at days 28-49 had no negative effects on body weight gain and feed utilisation [Skinner *et al.* 1992]. Some authors [Summers and Leeson 2005, Ruiz and Harms 1990] concerning the effects of short-term removal of vitamins and/or trace minerals agree that it is possible to exclude these nutrients at certain stages of fattening with no negative effects observed. However, the same authors emphasize that the time period may depend on temperature. It is unlikely that the concentration of trace minerals in edible parts of chicken meat is reduced as a consequence of 7-day removal of trace minerals from the chicken diet [Baker 1997]. Researches of other authors [Maiorka *et al.* 2002], where vitamins and trace minerals were removed from the diet from day 42 and 49 showed that there was no influence on body weight when both of them were removed, but feed conversion rate deteriorated in chickens fed a diet with no vitamins added. Liver mass was reduced when both vitamins and trace minerals were eliminated, but it had no negative effects on the carcass, leg and breast weight. Removal of only vitamins had more negative effects on feed conversion rate than that of minerals alone. The results from a study by Khajali *et al.* [2006] suggest that 14-day withdrawal of vitamins or trace mineral mixes did not influence the immune competence of broilers. Removing a vitamin premix from the broiler diet at day 29 of life had no negative effect on the immune system of chickens [Alahyari-Shahrab *et al.* 2011]. A study of Charuta *et al.* [2013a] showed that in a group of males reducing relative bone mass from 1.03 to 0.79% during the post-hatching development may lead to deformities and fractures of the tibia, which could have negative effects on broiler production.

The objective of this study was to determine the length of vitamin and trace mineral removal from the diet, i.e. how long their reserves in the body may be used without threatening growth intensity, as well as feed conversion rates and chicken health. It was assumed that sufficient amounts of trace minerals and fat-soluble vitamins would be stored in the body. Great quantities of water soluble vitamins cannot be generally accumulated, but chickens will continue to use them from natural feed, after exclusion of premixes.

Material and methods

The experiment was set up in the building for chicken fattening, on 1400 one-day old, Cobb 500 hybrid chicks, divided into seven groups of 200 animals each. Chickens were fed with two mixtures, which nutrient contents were compatible with the recommendations of hybrid line producers. One mixture (PI) was identical for all chickens and it was used for the first 15 days. Afterwards, up to the 30th day of fattening another mixture was used (PII), which also was the same for all the groups. From day 30 the same mixture as for days 15-30 was used, but either vitamins or trace minerals, or both were removed. After day 30, the following mixtures were used: 1) full premix at a concentration of 1% with vitamins and trace minerals according to the requirements of these animals (the control); 2) a mixture with 0.5% premix, with only trace minerals (NV); 3) a mixture with no premix, with no vitamins or trace minerals (NVM). The control group of chickens was fed with a full mixture, with vitamins and trace minerals up to day 42. The NV mixture was given to groups: 2 (30-42 day), 3 (34-42 day) and 4 (38- 42 day). The NVM mixture was used with the following groups: 5 (30-42 day of life), 6 (34-42 day) and 7 (38-42 day). At day 30 each chicken was marked with a leg ring and the groups were standardized as per mass and sex. All health measures were implemented as per the veterinary program. Body weight was recorded on each, every 7 days until day 30; subsequently body weight was recorded every 4 days, as supplements of vitamins and trace minerals were removed from the diet (30, 34, 38 and 42 day). At each measurement point, feed consumption was recorded and on this basis feed intake and feed conversion rates were calculated. Mortality of chicken was monitored and at the end of the production process the success index of fattening was calculated. The production index as an indicator of success of broiler fattening was calculated based on the broiler body weight, the percentage of surviving broilers, the length of the fattening period and feed conversion rates. It was calculated using the following formula:

$$\text{Production index} = \frac{\text{broiler body weight (kg)} \times \text{surviving broilers (\%)}}{\text{fattening period (days)} \times \text{feed conversion (kg)}} \times 100$$

After the completion of fattening, 10 chickens were sacrificed from each group (5 male + 5 female) and ash content in the leg bone was analysed. Also tibia strength was tested on an INSTRON 1122 strength testing machine. The data were subjected to the analysis of variance using the General Linear Model (GLM) procedure of the statistical

package programmes – STATISTICA 10 (2010). Since the complete diet was given to only one group for the entire period (control), and NV and NVM mixtures were given in three different periods: 4, 8 and 12 days, it was not possible to conduct data processing using only one model of variance. Instead, this was done separately using two ANOVA models, 3 X 2 (three mixtures, two sexes) and 2 X 3 X 2 (diet with no vitamins; diet with no vitamins, but with trace minerals; three removals in different time periods of 12, 8 and 4 days; two sexes).

Results and discussion

Basic indicators showing the effects of removal of vitamins and trace minerals in the final phases of chicken fattening were the most important natural parameters of production: chicken body weight, daily weight gain, feed intake, feed conversion rate and mortality. Based on these parameters, the production index was calculated.

Body weight

Chicken body weight did not differ considerably in later measurements in the first four weeks, i.e. until day 30 when we started the removal of vitamins and trace minerals. These results were expected, having in mind that until that period all chickens were treated identically. On day 30 of fattening, each chicken was marked with a leg ring with numbers from 1-200. Chicken groups were standardised by sex and body weight. The average mass of chickens in groups was 1180-1200 g for females, and 1250-1275 g for males. After 30 days chickens were fed mixtures with different contents of vitamins and trace minerals. In groups 2, 3 and 4 vitamins were removed from the premix on days 30, 34 and 38. In groups 5, 6 and 7 in the same time intervals both vitamins and trace minerals were excluded. At the end of the experiment, on day 42, body weight did not significantly differ between the groups, while statistical analysis showed that the effects of tested treatments had no significant impact (Tab. 1 and 2). Despite the small and statistically non-significant effect of tested dietary treatments on body weight, differences between male and female chickens were statistically highly significant.

Table 1. Average final body weight of chickens (g) fed with different mixtures of concentrates, monitored depending on sex

Type of diet (A)	Sex (B)		Average body weight
	male	female	
Complete diet (control)	2052.04	1891.91	1971.98 ^A
No vitamins (NV)	2049.48	1886.53	1968.01 ^A
No vitamins and trace minerals (NVM)	2042.53	1881.48	1961.97 ^A
Sex (Average body weight)	2048.02 ^A	1886.64 ^B	
p-values			
A		B	AB
0.746		≤0.01	0.916

^{AB}Within column means bearing different superscripts differ significantly at $P \leq 0.01$.

Table 2. Average final body weight of chickens (g) fed mixtures with no vitamin and minerals for 12, 8 and 4 days

Length of removal (B)	Sex (C)	Type of feed (A)		Average		
		No vitamins (NV)	No vitamins and trace minerals (NVM)			
12 days	male	2054.38	2042.41	1968.98 ^A		
	female	1891.09	1888.05			
	average	1972.74	1965.23			
8 days	male	2060.37	2049.58	1964.71 ^A		
	female	1881.91	1866.98			
	average	1971.14	1958.28			
4 days	male	2033.74	2035.54	1961.28 ^A		
	female	1886.54	1889.28			
	average	1960.14	1962.41			
Type of feed		1968.01 ^A	1961.97 ^A			
Sex		male 2046.00 ^A	female 1883.98 ^B			
p-values						
A	B	C	P (AB)	AC	BC	ABC
0.671	0.721	<0.001	0.643	0.696	0.303	0.098

^{AB}Within column means bearing different superscripts differ significantly at $P \leq 0.01$.

Having in mind the costs of feed and continuous studies to determine how to reduce those costs, as well as increasing consumer demand for products with fewer additives, organic products [Marzoni *et al.* 2014], removal of premixes from the diet of chickens in the last days of fattening would be advisable. In their study Wang *et al.* [2008] set up an experiment, in which they gave mineral premixes to chickens at 100, 80, 60, 40, 20 and 0% when compared to the recommendations. The conclusion was that a complete removal of mineral premixes leads to significantly lower final body weight of chickens, while chickens fed with 20% recommended premixes showed no differences in production parameters when compared with those fed with 100% premixes.

Feed intake, weight gain and feed conversion rates

Daily weight gains in all groups during the treatment ranged from 64.82 g to 67.8 g. Daily feed intake per chicken was 124.35 g to 139.89g. Feed conversion rate during the treatment was 1.89-2.14. Chickens subjected to the removal of vitamins and trace minerals for only four days had a feed conversion rate of 1.89-1.91. The ones with no vitamin and mineral supplements in their diet for 8 and 12 days had worse feed conversion rates, amounting to 10.14-16.33% when compared to the control group. If we consider the entire period of fattening, the best conversion rate was observed for chickens of the

control group, amounting to 1.86, and the groups with no additives in the last 4 days, 1.872 and then 1.886. The worst feed conversion rate was recorded for chickens fed the diet with vitamin and mineral supplements removed for the last 8 days, at 1.985 (6.7% worse than the control group), in comparison to chickens with removed vitamins and trace minerals for 12 days, 1.976 (6.2% worse than the control group). In terms of feed conversion rates in this study, in the period from the 30-42 day, they deteriorated in chickens fed a diet with the removal of vitamins for 8 and 12 days before the end of fattening, and in chickens with the removal of vitamins and trace minerals, both for 8 and 12 days. Feed conversion rates of chicken fed diets with these additives eliminated for only 4 days before the end of fattening were similar to those in the control group. Removal of both vitamins and trace minerals or only vitamins from the 42 to 49 day had no negative effects on feed intake and body weight of chickens [Spurlock *et al.* 1998]. However, the same authors indicated that the removal of only vitamins had more negative effects on feed conversion rates and weight gain than the removal of only minerals in the last stages of fattening in broilers. The results showed that the removal of only vitamin additives had significantly worse results on final body weight of chickens than the removal of only minerals, but including vitamin supplementation [Kim *et al.* 1997]. The same trend was observed for feed conversion rates. Those authors rejected the possibility of removal of vitamin and vitamin-mineral additives for 21 days with no negative effects on production results in chicken fattening. Authors of several papers [Patience *et al.* 1995, Ruitz *et al.* 1990] concerning consequences of short-term removal of vitamins and/or trace minerals agree that it is possible to eliminate these nutrients with no bad effects.

Mortality of chickens

During the entire period of chicken fattening, mortality was identical in all groups, amounting to mean 1.93%.

Production index

Based on all the above-mentioned parameters, production indexes were calculated for each chicken group in order to compare results of fattening (Fig. 1). The production index is an indicator of broiler fattening success. It was calculated based on the broiler body weight, the percentage of surviving broilers, the length of the fattening period and feed conversion rates. The best production index was found in groups 4 (4 days with no vitamins), 240.5, followed by 7 (4 days no vitamins + minerals), 239.1, and the control at 237.3. The worst production indexes were recorded in the groups with 12-day removal of vitamins, 221.9, and 12-day removal of vitamins + trace minerals, 228.5. This means that the 4-day exclusion of only vitamins or both minerals and vitamins had no adverse effect on the production index. On the other hand, the 8- and 12-day exclusion of these substances negatively affected the production index.

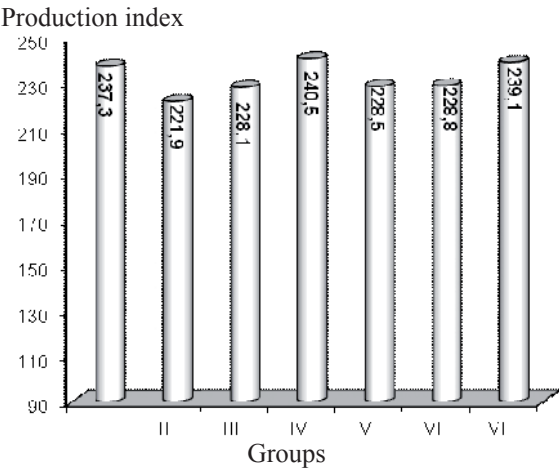


Fig.1. Production indexes depending on groups

Slaughtering parameters

Slaughtering parameters were determined for the following body parts: drumsticks, thighs, breasts, backs, wings, legs, necks, edible entrails and abdominal fat. Statistical analysis confirmed that slaughtering parameters showed no significant differences between the types of mixtures in terms of the length of their removal from the diet, except for abdominal fat. Identical percentages of carcass parts can be observed in Figure 2.

Figure 3 presents values of edible entrails, neck and abdominal fat mass.

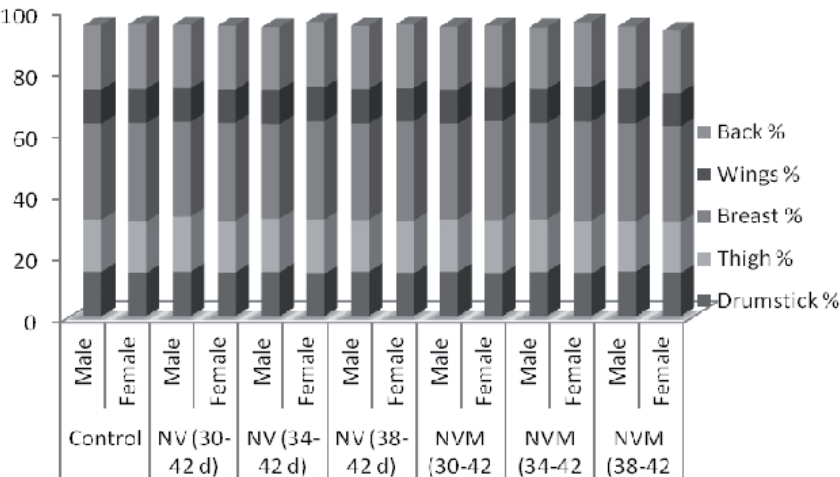


Figure 2. Percentage shares of carcass parts

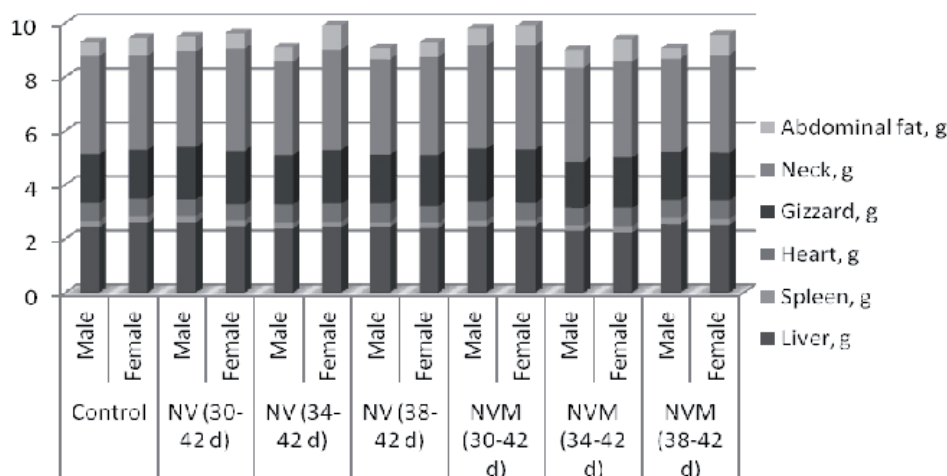


Fig. 3. Values of edible entrails, neck and abdominal fat mass.

However, highly significant differences were found for each slaughtering parameter, with males having higher values for all parameters, except for abdominal fat. Statistical analysis for the parameter of abdominal fat (in animals fed with different mixtures of concentrates, monitored depending on sex) is shown in Table 3.

Table 3. Average mass of abdominal fat (g) of chickens fed with different mixtures of concentrates, depending on sex

Type of diet (A)	Sex (B)		Average
	male	female	
Complete diet (control)	10.70	12.60	11.65 ^A
No vitamins (NV)	9.97	12.37	11.17 ^A
No vitamins and trace minerals (NVM)	11.67	14.63	13.15 ^A
Sex (Average body weight)	10.77 ^A	13.27 ^B	
p-values			
A	B		AB
0.524	≤0.004		0.284

^{AB}Within column means bearing different superscripts differ significantly at $P \leq 0.01$.

It is evident that male chickens had significantly less abdominal fat than female chickens. Table 4 shows the data for the same parameter, depending on the duration of vitamin or vitamin and mineral removal.

The results of the t-test showed that the 4-day exclusion of vitamin or vitamin and mineral supplements resulted in the formation of the smallest amount of abdominal fat. This is a statistically significant difference from the abdominal fat mass recorded for the 12-day exclusion of vitamin and mineral supplements.

Table 4. Average mass of abdominal fat (g) of chickens fed mixtures with no vitamin or mineral supplements added for 12, 8 and 4 days

Length of removal (B)	Sex (C)	Type of feed (A)		Average		
		No vitamins (NV)	No vitamins and trace minerals (NVM)			
12 days	male	11.20	13.10	12.07 ^{AB}		
	female	10.30	13.70			
	average	10.75	13.40			
8 days	male	10.40	13.90	14.15 ^A		
	female	16.70	15.60			
	average	13.55	13.65			
4 days	male	8.30	8.00	10.25 ^{BC}		
	female	10.10	14.60			
	average	9.20	11.3			
Type of feed		11.17 ^A	13.15 ^A			
Sex		male 10.82 ^B	female 13.50 ^A			
p-values						
A	B	C	AB	AC	BC	ABC
0.336	<0.001	0.003	0.280	0.140	0.617	0.354
The differences between means of exclusion length of vitamin and trace mineral supplements						
12 days 12.07 g (X ₁)			t ₁₋₂		1.63	
8 days 14.15 g (X ₂)			t ₁₋₃		1.42	
4 days 10.25 g (X ₃)			t ₂₋₃		3.05 ^{**}	

^{AB}Within column means bearing different superscripts differ significantly at $P \leq 0.01$.

Ash content in chicken bone

Ash content was determined in leg bones of chickens, with the results depending on different feed mixtures and sex shown in Table 5.

Data in Table 5 show a statistically significant effect of sex on the tested parameter, as well as a statistical significant effect of the type of feed. Chickens fed with the complete diet had the greatest ash content in leg bones at 52.09%; chicken fed with the NVM mixture had ash content of 51.92%, while the lowest content (51.09%) was recorded for chickens with no vitamin supplements added to their diet (NV). Thus it may be concluded that feeding chickens the complete diet had the best effect on ash content in bones, while feed with no vitamins added reduced it the most. This could be explained by the fact that the presence of trace elements led to an increase in the oxidation of vitamins found in the feed and thus adversely affect ash content. No interactions between these two factors were found. Results for the same parameter in terms of the duration of removal of vitamins and minerals, type of mixtures and sex, are shown in Table 6.

Table 5. Ash contents in leg bones (%) of broilers fed with different mixtures of concentrates, monitored depending on sex

Type of diet (A)	Sex (B)		Average
	male	female	
Complete diet (control)	52.11	52.06	52.09 ^A
No vitamins (NV)	50.41	51.77	51.09 ^B
No vitamins and trace minerals (NVM)	51.40	52.44	51.92 ^{AB}
Sex	51.31 ^B	52.09 ^A	
p-values			
A	B		AB
0.000	0.002		0.290
The differences between means of the mixtures in chicken feeding			
Complete diet	52.09 %	t ₁₋₂	2.93**
NV mixture	51.09 %	t ₁₋₃	0.50
NVM mixture	51.92 %	t ₂₋₃	2.43*

^{AB}Within column means bearing different superscripts differ significantly at $P \leq 0.01$.

Table 6. Ash contents in leg bones (%) of broilers fed with concentrates with no vitamin and mineral supplements added for 12, 8 and 4 days

Length of removal (B)	Sex (C)	Type of feed (A)		Average		
		No vitamins (NV)	No vitamins and trace minerals (NVM)			
12 days	male	49.12	51.28	50.54 ^{CB}		
	female	49.50	52.26			
8 days	male	50.69	50.04	51.57 ^{BA}		
	female	53.19	52.34			
4 days	male	51.43	52.88	52.42 ^A		
	female	52.62	52.73			
Type of feed		51.09 ^A	51.92 ^A			
Sex		male	female			
		50.91 ^B	52.11 ^A			
p-values						
A	B	C	AB	AC	BC	ABC
0.098	0.002	0.000	0.003	0.068	0.102	0.099
The differences between means of exclusion length of vitamin and trace mineral supplements						
12 days	50.54 %		t ₁₋₂		2.46*	
8 days	51.57 %		t ₁₋₃		4.49**	
4 days	52.42 %		t ₂₋₃		2.03*	
Interaction effects						
Type of mixture X and length of removal of vitamins and minerals						
		12 days		8 days		4 days
NV mixture		49.31		51.94		52.03
NVMmixture		51.77		51.19		52.81

^{ABC}Within column means bearing different superscripts differ significantly at $P \leq 0.01$.

The t-test for the significance of differences between the lengths of removal of vitamins and minerals shows the following: 1) ash content in leg bone during the

removal of vitamins and minerals from the diet in the last 12 days of fattening showed statistically significant differences in comparison with the data for the 8-day removal; 2) ash content in leg bone during the removal of vitamins and minerals in the last 4 days of fattening showed statistically significant differences in comparison with ash content for the 12-day removal and for 8-day removal. Moreover, a significant interaction was observed for the type of feed and the duration of removal of vitamin and mineral additives.

Strength of tibia bone

Statistical analysis showed that the effects of tested treatments did not have any significant on tibia strength (Tab 7). In spite of a lack of statistically significant differences, a lower bone resistance was observed only in males. A study of Charuta *et al.* [2011] on the Peking domestic duck showed that the number of trabeculae differed significantly depending on the age, sex, and specific fragments of the studied bone. Six-week-old hens, which tibiotarsal bones were most often exposed to deformities and fractures, had a reduced bone mass. Another study by Charuta *et al.* [2014] conducted on geese showed that bone deformations and fractures in females occur slightly later than in males. Charuta *et al.* [2012] performed investigations also on turkeys. Those researchers concluded that volumetric bone mineral density in the diaphyses gradually attenuated with age for both sexes, whereas in the metaphyses it was constant in females, whereas in males it reached the maximum value at weeks 6 and 12 and the minimum at weeks 9 and 16, respectively. A study by Charuta *et al.* [2013b] conducted on quails showed that a decrease of volumetric bone mineral density and mineral contents in the metaphyses of tibiotarsal bones in males occurred in the 4-6 week of the post-hatching development, with the lowest values of these parameters observed in week 6. On the other hand, in females the decrease of these parameters was recorded in the 4th and 8th weeks. All the above-mentioned studies suggest that males are more prone to limb problems than females. This is demonstrated in this paper, where males have a lower bone resistance due to the lack of vitamins and minerals.

Table 7. Strength of tibia bone (N/cm²) of broilers fed with different mixtures of concentrates, monitored depending on sex

Type of diet (A)	Sex (B)		Average
	male	female	
Complete diet (control)	3162.36	3057.46	3334.91 ^A
No vitamins (NV)	2844.44	3063.02	2953.73 ^A
No vitamins and trace minerals (NVM)	2700.00	2867.20	2783.58 ^A
Sex (Average strength of tibia bone)	2903.37 ^A	3211.17 ^A	
p-values			
A	B		AB
0.122	0.088		0.210

^{AB} Within column means bearing different superscripts differ significantly at $P \leq 0.01$.

Final body weight did not significantly differ between groups. While statistical analysis showed no significant effects of the tested treatments, but differences between male and female chickens were statistically highly significant. Chickens subjected to the removal of vitamin and vitamin + trace mineral supplements from the diet for the last 8 and 12 days of fattening had worse feed conversion rates, 4.14-5.16% lower than the control. Feeding chickens with the complete feed mixture had the best effect on ash content in bones, while feed with no vitamin supplements reduced the value of that parameter the most. Most probably the presence of trace minerals led to increased oxidation of vitamins in the feed and therefore negatively influenced production characteristics. The most depressive effect on ash content in leg bones was observed when vitamin additives were removed 12 days before the end of fattening. Statistical analysis showed that mixtures with no vitamins and trace minerals added had no negative effects on slaughtering parameters and tibia strength. In spite of a lack of statistically significant differences, a lower bone resistance was observed only in males. In some cases, no negative effects of removing supplements of vitamin and trace elements from the diet were recorded. However, bearing in mind the technological processes of broiler chicken production, animal welfare (problems with legs), and the importance of poultry meat in human diet (as a source of vitamins and minerals), we can conclude that removing of vitamins and trace minerals from broiler chicken diets in the final stages of fattening is not entirely advisable.

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