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Effect of different amounts of pansy and nettle in feed mixtures for chickens on physical, chemical and strength parameters of their bones

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Summary

The aim of the present study was to determine the effect of an herbal supplement used in feed mixtures on the selected physical features and the chemical composition of broiler chickens' bones in reference to antibiotic growth promoter. The experimental material was provided by 180 ROSS broiler chickens. One-day-old nestlings were divided randomly into 2 control groups and 4 experimental groups, in three replications, 10 birds each. The feed mixtures (Starter and Grower/Finisher types) were made of corn and extracted soy meal and they also contained fodder yeast, soy oil, mineral additives, premixes (with an addition of growth promoters or without) and an addition of dried green forage or dried herbs. The mechanical properties of the bones were determined on the basis of the three-point bend test performed with the use of an Instron 4302 apparatus coupled with a computer. The test registers as a graph the dependence between the force acting perpendicularly to the long axis of the bone and its deformation. An examination was performed to determine maximum force, and the maximum elasticity force. The studies revealed that both the type and the amount of the herbs determined chickens' growth and the physical and strength properties of their bones. The most beneficial effects were observed in feeding birds mixtures containing 1% of pansy, since the values noted for all traits and indices were not worse than the effects obtained in the control group receiving antibiotic growth promoter, and in some cases they were even higher. The least positive results occurred in supplementing feed mixtures with 3% of nettle.

Keywords: broilers, herbs, femoral bones, physical, elastic and chemical parameters

The bone-building process is a result of a number of factors, and primarily of hormones, vitamins and indispensable minerals. One of the reasons why this process is disturbed in chickens is their high growth rate which, while being adequate for the consumed energy and protein, may be too quick in relation to the birds' ability of absorbing calcium and other mineral elements (15). A change in the proportion of muscle tissue to bone tissue in poultry leads to an excessive load or overload and thus fractures of their pelvic bones and, consequently, to disturbances of their growth and skeleton mineralization and, simultaneously, lower efficiency of performance (11, 19, 20). During meat processing following slaughter weakened bones of the limbs are easily damaged, which reduces the quality of carcasses. Another important issue is the function of the bone system as a place for storing minerals and thus its vital role in regulating electrolyte concentration in the organism (14).

Among feed additives having a potentially beneficial effect on the effectiveness of poultry production, herbs are considered as a replacement for antibiotic growth promoters (4, 5). The advantages of such plant additives are a lack of side effects, safety of animal products and their multi-dimensional effect on the animal's organism. Among others, active substances present in herbs regulate digestive functions, stimulate the organism's metabolism, have an immunomodulatory function and so contribute to animals' higher performance and better health (6, 8-10, 13). Among numerous studies on the use of herbs in poultry rearing there are few papers concerning the role of herbs in the skeletal system.

The leaves of the nettle (*Urtica dioica*) contain carotenoids, vitamins C, K and B_2, pantoten acid, chlorophyll, xantophyll, tannins, flavonoids and minerals, such as calcium, phosphorus, magnesium and iron, as well as organic acids. Pansies (*Viola tricolor*) contain,

among others, saponins, flavonoids (including rutin and quercetin), anthocyanins, mucous substances, carotenoids, vitamin C, tannins, salicylic acid and caffeic acid. Thus, the bioactive substances present in the nettle or pansy added to dietary portions may have a positive effect on the course of metabolic processes, as well as they may increase the level of calcium in the blood and bones.

The aim of the present study was to determine the effect of a herbal supplement used in feed mixtures on the selected physical features and the chemical composition of broiler chickens' bones in reference to an antibiotic growth promoter.

Tab. 1. Experimental design

Group	Feed additive
K-1	premix with antibiotic, 1% dried herbs
K-3	premix with antibiotic, 3% dried herbs
P-1	premix without antibiotic, 1% pansy (*Viola tricolor* L.)
P-3	premix without antibiotic, 3% pansy (*Viola tricolor* L.)
N-1	premix without antibiotic, 1% nettle (*Urtica dioica* L.)
N-3	premix without antibiotic, 3% nettle (*Urtica dioica* L.)

Material and methods

The experimental material was provided by 180 ROSS broiler chickens. One-day-old nestlings were divided randomly into 2 control groups and 4 experimental groups, in three replications, 10 birds each. The birds were reared in a battery cage system in standard environmental conditions of an experimental poultry house for 41 days.

The chickens were fed *ad libitum* with pelleted feed mixtures not containing any material of animal origin. The feed mixtures (Starter and Grower types) were made of corn

Tab. 2. Composition (%) and nutritive value of the mixtures

Components	Starter				Grower/Finisher			
	Groups							
	K-1[a]	K-3[a]	P-1, N-1	P-3, N-3	K-1[b]	K-3[b]	P-1, N-1	P-3, N-3
Maize	55.5	53.5	55.5	55.5	59.0	57.0	59.0	59.0
Soybean meal 46%	33.0	33.0	33.0	33.0	28.0	27.5	28.0	28.0
Yeast	3.0	3.0	3.0	3.0	3.0	3.0	3.0	3.0
Dried grass silage meal	1.0	3.0	–	–	1.0	3.0	–	–
Herbs meal**	–	–	1.0	3.0	–	–	1.0	3.0
Soya oil	3.0	3.0	3.0	3.0	4.0	4.5	4.0	4.0
Dicalcium phosphate	1.5	1.5	1.5	1.5	2.0	2.0	2.0	2.0
Limestone	1.5	1.5	1.5	1.5	1.5	1.5	1.5	1.5
NaCl	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5
Premix with antibiotic*	1.0	1.0	–	–	1.0	1.0	–	–
Premix with antibiotic*	–	–	1.0	1.0	–	–	1.0	1.0
1 kg feed mixtures contains:								
ME (MJ)	12.80	12.65	12.80	12.80	13.09	13.06	13.09	13.09
Crude protein (g)	209	204.4	216.6	218.4	194.9	194.1	196.8	196.3
Crude fibre (g)	31.8	36.4	32.0	36.0	31.9	34.6	30.9	34.8
Ca (g)	10.18	10.18	10.18	10.18	9.47	9.47	9.47	9.47
P total (g)	6.97	6.97	6.97	6.97	6.29	6.29	6.29	6.29
Mg (g)	1.94	1.94	1.94	1.94	1.72	1.72	1.72	1.72
Lysine (g)	12.12	12.12	12.12	12.12	9.98	9.98	9.98	9.98
Met + Cys (g)	7.77	7.77	7.77	7.77	7.71	7.71	7.71	7.71

Explanations: K-1, K-3 – control group; P-1, N-1, P-3, N-3 – experimental groups; S – starter mixture; G/F – grower/finisher mixture group;

a – Composition of premix starter/kg: vit. A 1 360 000 IU, vit. D_3 325 000 IU, vit. E 4000 mg, vit. K3 250 mg, vit B_1 200 mg, vit. B_2 600 mg, vit. B_6 300 mg, vit. B_{12} 2000 µg, biotin 15 mg, folic acid 125 mg, nicotinic acid 3500 mg, D-calcium panthotenate 1200 mg, choline chloride 60 000 mg, Mn 9000 mg, Cu 2000 mg, Zn 10 000 mg, Fe 9000 mg, J 100 mg, Se 25 mg, Co 70 000 µg;

b – Composition of premix grower/finisher/kg: vit. A 1 200 000 IU, vit. D_3 300 000 IU, vit. E 3000 mg, vit. K3 200 mg, vit B_1 150 mg, vit. B_2 500 mg, vit. B_6 250 mg, vit. B_{12} 1500 µg, biotin 10 mg, folic acid 100 mg, nicotinic acid 2500 mg, D-calcium panthotenate 1000 mg, choline chloride 60 000 mg, Mn 9000 mg, Cu 2000 mg, Zn 10 000 mg, Fe 9000 mg, J 100 mg, Se 25 mg, Co 70 000 µg;

* antibiotic Flavomycin (0.1%);

** the share of herbs in accordance with the methodical system: P-1, N-1 – pansy, nettle – 1%, P-3, N-3 – pansy, nettle – 3%

and extracted soy meal; they also contained fodder yeast, soy oil, mineral additives, premixes (with an addition of growth promoters or without) and an addition of dried green forage or dried herbs (tab. 1). Composition and nutritive value of the control and experimental mixtures are presented in table 2.

In the present study we used herbs delivered by Herbapol Lublin PLC. The herbs had been classified as waste only due to their excessive refinement, rather than because of their biological value that was identical to that of the raw material on the market, which was confirmed by the research conducted by the laboratory of Herbapol Lublin PLC. All herbal waste was free from any organic or mineral contamination.

After weighing chickens on day 42, 8 birds from each group, about the average weight, were sacrificed and subjected to dissection. Selected broiler chicken were subjected to fasting for 10 hours. Subsequent to knocking them unconscious, they were slaughtered by decapitation.

During the dissection analysis, their femoral bones were taken for preparations according to the Polish method described by Ziołecki and Doruchowski (21).

The bones were mechanically separated from soft tissues and their length was measured with the use of an electronic caliper (to the nearest 0.001 mm), each time with the settled position of the bone during the measurement. Their circumference was measured at ″ of the bone's length after which they were packed into labeled foil bags and frozen (at –25°C) until the moment of starting the analyses.

The weight, length and percentage share of the bone in the total body weight were assessed.

The mechanical properties of the bones were determined on the basis of the three-point bend test performed with the use of Instron type 4302 apparatus load coupled with a computer. The test registers as a graph the dependence between the force acting perpendicularly to the long axis of the bone and its deformation (6). The assessment criterion was loading force at constant speed of the measuring head $V = 10\ mm \cdot min^{-1}$. The span of supports constituted 40% of the total length of the analyzed bones. An examination was performed to determine maximum force, which is also known as the breaking point, at which the bone is disintegrated and its structure is broken, and the maximum elasticity force, known as the elasticity point. When the elasticity point is exceeded, the bone reacts like a plastic substance.

On the basis of the performed measurements the following strength parameters of the bones were determined: elasticity value – Wy, the value of the bending moment of maximum force $Wf = Wc \times L$, where: Wc – maximum force, L – width of supports' span in bending test; dy – bone disintegration with elasticity limit exceeded, the so-called deflection; Wy/dy – rigidity as a ratio of the force acting on the bone to the extent of bone disintegration provoked by this force; Wf/A – bone resistance to bending, determined as the ratio of the force acting on a unit of the surface of shaft's transverse cross-section, where A – surface area of transverse cross-section.

The content of crude ash was determined with the use of the dry mineralization method at 650°C. After the incineration process at a temperature of 650°C, crude ash and basic mineral components Ca, Mg and P were determined. Crude ash was determined by the AOAC method (2).

The content of Ca and Mg was determined with the use of atomic absorption spectrophotometry on ASA SOLAR 939 UNICAM flame spectrophotometer, according to PN – EN ISO 6869: 2002, and unlimited P on a Helios Delta apparatus was measured – using spectrometric method according to PN – ISO 6491: 2000.

The results were subject to a statistical analysis with the use of the Statistica 5.0 program. The analysis of variance was performed and the significance of differences was verified with T-Tukey's confidence intervals.

Results and discussion

The type and amount of a growth stimulating additive in feed mixtures conditioned the final body mass of broiler chickens. The most effective was the addition of 1% pansy. The body mass of the chickens in K-1 group (antibiotic and 1% of dried green forage) and P-1 group (1% of pansy) was higher than in K-3 (antibiotic and 3% of dried green forage), P-3 (3% of pansy) or N-3 (3% of nettle). The differences were statistically confirmed (tab. 3).

Femoral bones in chickens from the K-1 group and those fed mixtures containing a 1% share of herbs (P-1, N-1) had a higher mass than the bones of the chickens in group K-3 and those receiving a 3% share of additives. The differences between mean values for the group were statistically insignificant. The bones

Tab. 3. Chickens' body weight and physical parameters of femoral bones

	K-1	K-3	P-1	P-3	N-1	N-3
Body weight, g						
M	2252.5^{c}	1885.6^{ab}	2313.7^{c}	1856.2^{ab}	2091.2^{bc}	1722.5^{a}
SD	359.67	336.06	346.49	169.28	272.89	169.52
Weight of femoral bones, g						
M	$8,86^{a}$	7.69^{a}	8.69^{a}	7.66^{a}	8.18^{a}	7.32^{a}
SD	1,55	1.29	1.18	0.68	0.85	0.54
Length of femoral bones, mm						
M	71.1^{b}	67.5^{a}	70.6^{b}	68.9^{ab}	69.7^{ab}	67.9^{a}
SD	2.75	3.07	2.87	2.25	1.81	1.73
Share of bones in body weight, %						
M	0.39^{a}	0.41^{a}	0.38^{a}	0.41^{a}	0.40^{a}	0.43^{a}
SD	0.044	0.062	0.032	0.034	0.051	0.045

Explanations: K – 1, 3 – control group; P, N – 1, 3 – experimental groups; M – mean; SD – standard deviation; a, b, c – means with different superscripts differ significantly at $p \leq 0.05$

with a higher mass were also longer, like in the studies performed by Szeremeta et al. (18).

The effect of adding 1% of pansy or nettle to feeding mixtures was a lower share of femoral bone in comparison with the effects brought about by adding 3% of such additives and the differences were not statistically significant. Reaching a lower share of the femoral bone in the body mass at a higher total body mass confirms the positive influence of using herbs in feed mixtures on the final body mass in chickens and suggests higher slaughter performance. In relation to the results of the studies done by Alçiçek et al. (1), who noted higher body mass and slaughter performance in chickens fed a higher share of herbal supplements, it can be claimed that the results may have been affected by a higher share of crude fiber in the mixtures containing 3% of the supplement. At the same time, bearing in mind a lack of any influence of using an extract with oregano on the body mass observed by Botsoglou et al. (3), the analyzed herbs, and especially pansy, may be regarded as useful in chicken feeding.

The nutritional versions used in the experiment modified bone strength expressed with the value of maximum strength (tab. 4). The bones in the chickens receiving a 1% supplement were stronger than the bones in the chickens fed mixtures with a 3% addition of the same component. The largest difference was noted between the bones in group N-3, where Wf amounted to 206.35 N·mm, and P-1, where it was higher by 32%.

There was a random differentiation in the value of elasticity force (Wy) and of mean values for the group. Similarly to the case of elasticity force value, nutritional versions did not affect the deformation when the elasticity limit was exceeded for the average value of the whole groups.

The proportion of elasticity force value to deformation at exceeding elasticity limit Wy/dy, or elasticity, was not significantly varied, despite relevant differences between the mean values for individual sexes (tab. 4). A comparison of the mean values for the group revealed that after using a 3% addition of pansy in a feed mixture the lowest rigidity was obtained, amounting to ca. 116 N/m. This particular value was significantly lower than in the bones in K-1, the difference was 54%. Just like in the case of several other properties of chickens' bones, in the „1%" group mean values were higher and more similar than the bones of the chickens in the „3%" group.

Bending moment resistance (Wf/A) was the third analyzed property (apart from body mass and bone length) in which statistically significant differences occurred between the mean values for the control groups, i.e. K-1 and K-3, amounting to 7.27 in reference to 5.04 N·mm/mm. The resistance of femoral bones in the chickens from the remaining groups was within the above mentioned limits.

Tab. 4. Strength parameters of broiler chickens' femoral bones: maximum force value (Wf), elasticity force (Wy), deformation at exceeding elasticity limit (dy), rigidity (Wy/dy) and bending point resistance (Wf/A)

	K-1	K-3	P-1	P-3	N-1	N-3
Wf, N•mm						
M	263.89^{bc}	217.17^{b}	272.71^{c}	236.14^{abc}	237.45^{abc}	206.35^{a}
SD	25.92	64.77	66.41	42.93	48.33	33.84
Wy, N						
M	167.82^{a}	146.22^{a}	200.19^{a}	145.75^{a}	163.90^{a}	149.17^{a}
SD	59.27	49.99	82.81	29.96	69.5	61.19
dy, mm						
M	1.11^{a}	1.21^{a}	1.29^{a}	1.30^{a}	1.02^{a}	1.20^{a}
SD	0.59	0.58	0.35	0.2	0.36	0.57
Wy/dy, N/mm						
M	178.27^{b}	129.34^{ab}	155.45^{ab}	115.89^{a}	170.16^{ab}	139.90^{ab}
SD	74.72	36.08	42.43	34.81	69.46	56.73
Wf/A, N•mm/mm						
M	7.27^{b}	5.04^{a}	5.64^{ab}	6.21^{ab}	7.25^{b}	5.81^{ab}
SD	2.67	1.72	1.45	1.21	2.18	0.96

Explanations: as in tab. 3

Tab. 5. Mineral composition of femoral bones in healthy broiler chickens

	K-1	K-3	P-1	P-3	N-1	N-3
Crude ash (%)						
M	45.18^{a}	50.96^{b}	47.43^{ab}	47.11^{ab}	46.86^{ab}	47.19^{ab}
SD	3.09	6.52	2.37	2.39	2.37	3.61
Ca (mg•kg^{-1})						
M	188.64^{a}	217.43^{b}	202.57^{ab}	206.35^{ab}	205.82^{ab}	211.22^{ab}
SD	21.66	16.15	27.27	11.75	6.88	9.36
P (mg•kg^{-1})						
M	125.56^{a}	144.56^{b}	133.14^{ab}	133.97^{ab}	136.12^{ab}	139.21^{ab}
SD	3.72	3.45	13.26	6.09	6.22	9.74
Mg (mg•kg^{-1})						
M	6.72^{a}	7.74^{a}	7.11^{a}	6.97^{a}	7.09^{a}	7.19^{a}
SD	0.44	0.89	0.69	0.54	0.82	0.57

Explanations: as in tab. 3

The share of mineral elements in bones fluctuated between 45.18 (K-1) and 50.96% (K-3) of the bone mass, and the extreme values differed significantly (tab. 5). In the bones of the chickens fed mixtures with herbal supplements the share of ash in the bone mass was similar regardless of the type and amount of herbs. The content of calcium and phosphorus was also differentiated between the femoral bones in the control groups. A higher amount of dried green forage, with a content of antibiotic growth promoter, significantly increased the concentration of Ca and P in the bones. The mixtures containing pansy or nettle, regardless of their share, resulted in similar levels of these elements. In case of magnesium, there were no significant differences concerning its content between the mean values for the group.

A possibility of modifying the properties of chicken bone tissue with the help of herbs was also noted when using a 2% supplement (12). The herb which increased the majority of physical and strength properties was nettle. However, adding other herbs, including pansy, to feed mixtures resulted in higher parameters in comparison with the control group.

The studies also revealed that both the type and the amount of the herbs determined chickens' growth and the physical and strength properties of their bones. The most beneficial effects were observed in feeding birds mixtures containing 1% of pansy, since the values noted for all traits and indices were not worse than the effects obtained in the control group receiving antibiotic growth promoter, and in some cases they were even higher. The least positive results occurred in supplementing feed mixtures with 3% of nettle.

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