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THE EFFECT OF GARLIC (*ALLIUM SATIVUM* L.) ON GROWTH PERFORMANCE, MORTALITY RATE, MEAT AND BLOOD PARAMETERS IN BROILERS*

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Abstract

The effect of garlic extract on body weight, feed intake, feed conversion rate, mortality rate, dressing percentage, carcass traits, chemical composition of breast muscles and blood plasma parameters was investigated in a feeding trial with 640 Ross 308 broiler chickens of both sexes. The experiment was characterised by a two-factor design, with liquid garlic extract (GFA) and sex as factors. In comparison with the unsupplemented control group, supplementing diets with 1.00 (GFA1), 1.50 (GFA2), and 2.25 (GFA3) ml kg^{-1} for 42 days increased body weight, with a significant difference for the GFA3 group (P≤0.01). Bird mortality was 2.78% in the control group and 0.10%, 0.63%, and 0.60% in the GFA1, GFA2, and GFA3 supplemented groups, respectively (P≤0.01). Feed intake (kg/42 days) was 4.50 per broiler in the control group and 4.51, 4.68, and 4.85 kg^{-1} per broiler in the experimental groups (P≥0.05), respectively. Feed conversion rate was 1.80 in the control group and 1.77, 1.76, and 1.80 kg/kg in the GFA1, GFA2, and GFA3 groups, respectively (P≥0.05). GFA increased European Production Efficacy Factor (EPEF) from 331 in the control group to 347, 363, and 356 points in the experimental groups, respectively (P≤0.01). Dressing percentage in the GFA3 group was significantly higher than in the control group (74.8% vs 72.5%; P≤0.01). GFA at the concentration of 2.25 ml kg^{-1} significantly increased the weight of breast muscles. Supplementing the diet with GFA at the level of 1.50 ml kg^{-1} caused a significant increase in liver weight (P≤0.01). Feeding with GFA at a rate of 1.5 ml kg^{-1} caused a significant increase in the protein and crude ash content of breast meat compared to the control group (P≤0.05). The highest dose of GFA significantly increased total protein content of serum compared to the control group (P≤0.01). No significant interaction of GFA by sex on the analysed parameters was found. It is concluded that GFA at 2.25 ml kg^{-1} caused a significant improvement in the body weight of broilers and their carcass parameters, while a significant reduction in bird mortality for the GFA-supplemented groups was observed during the rearing period. It appears that the optimum level of GFA, when the crude protein concentration in the commercial starter and grower diets ranges between 210 and 220 g kg^{-1} is 1.5–2.25 ml kg^{-1} of the starter and grower diets.

Key words: broiler chickens, garlic, body weight, mortality, feed utilisation, meat and serum parameters

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A ban on the use of antibiotics apart from medical feeds was imposed by the European Commission in January 2006, following confirmation that some bacterial strains were resistant to antibiotics used in humans and animals (EC Regulation No. 1831/2003). This fact has triggered research on natural feed additives that exhibit antibacterial properties and protect the avian digestive tract against various pathogenic bacteria. In the digestive tract of birds, bacteria originating from feed, water and the environment can induce inflammatory processes, especially in the small intestine, thus impairing nutrient absorption, reducing chicken growth rate and productivity, and lowering immune resistance, as well as causing necrotic enteritis, which damages the mucosa by destroying the intestinal villi (Bedford, 2000; Annett et al., 2002; Dahiya et al., 2006). In extreme cases, bacteria can induce intestinal mucosal inflammation leading to excessive diarrhoea and death. Previous studies have already shown that human nutrient supplements and feed additives derived from garlic possess antibacterial properties (Lanzotti, 2006; Toghyani et al., 2011; Negi, 2012; Peinado et al., 2012, 2013). Garlic was recognised as a valuable component of the human diet over 5,000 years ago in Egypt, Greece, China and India (Milner, 2001). It exerts health-promoting effects by preventing the development of bacteria, such as *Escherichia coli*, *Enterobacteria* Spp., and *Salmonella typhimurium* (Kumar and Berwal, 1998; Ross et al., 2001). Medical research on humans and experiments with rats and poultry have confirmed that garlic lowers blood levels of LDL cholesterol and reduces the rate of cholesterol oxidation (Yeh and Liu, 2001; Lau, 2001), displays antioxidant and anti-cancer activity (Borek, 2001; Milner, 2001; Yang et al., 2001), and enhances the immune resistance of living organisms (Kyo et al., 2001). Garlic contains an active ingredient called alliin, which, when garlic is crushed in aerobic conditions, is converted by the enzyme allinase into allicin (Lanzotti, 2006). The intermediate compound is alkyl sulphonic acid, which has the capacity to acidify the digesta of animals, and the sulphides released from allicin exert strong antibacterial and antioxidant activity (Sallam et al., 2004; Lanzotti, 2006; Bozin et al., 2008). Allyl sulphides exert multiple, antifungal, anti-inflammatory and immune-enhancing activity and can regenerate liver tissue (Amagase et al., 2001; Tatara et al., 2005; Kandil et al., 1987). In animal production, garlic is usually used in the form of crushed bulbs, powder, garlic oil, extracts, and in mixtures with other herbs, mainly thyme (Puvača et al., 2013). Garlic extracts are considered much more potent than formic acid. Allivet liquid garlic, which is available on the Polish market, should be used twice a week or three times in a row in a three-week cycle at concentrations of 1.0 ml kg^{-1} of a poultry commercial mixture. This additive can be added either to feed or to drinking water at the level of 1.00 ml kg^{-1}. Another option is to administer the additive in feed on a regular basis, but in this case no dosage has been specified. With the intention of finding the optimal level of this garlic-based additive dietary supplementation used on a regular basis, the recommended Allivet dose of 1.00 ml kg^{-1} of feed was compared to a diet without Allivet and to diets with the above Allivet dose increased by 50% or 125%.

The objective of the study was to determine the effect of different doses of the GFA Allivet (1.00; 1.50 and 2.25 ml kg^{-1}) compared to an unsupplemented control group on growth performance in broiler chickens of both sexes, including body

weight, mortality rate, feed conversion rate, carcass traits, composition of breast muscles, and blood parameters.

Material and methods

Animals, experimental design, diets and treatments

The experiment was approved by the Local Ethics Committee in Kraków, Poland. A total of 640 sexed Ross 308 broiler chickens with an initial body weight of 45±7 g, in a 4 × 2 design, were randomly allotted to 4 groups (GFA factor), each of which was divided into 2 subgroups (sex factor). Liquid garlic extract (GFA) was added to feed when mixing feed components at levels of 0.00 (control group), 1.00, 1.50, and 2.25 ml kg^{-1} (experimental groups). The GFA additive is a water solution of extract of garlic bulbs, distributed by the Centaur Company (Poland). These amounts of the mixture were intended for specific groups of broilers and particular stages of rearing. For each subgroup there were 8 replications with 10 broilers each. Chickens were fed *ad libitum* with complete starter (days 1–21) and grower (days 22–42) diets in accordance with the Nutrient Requirements of Poultry (Poultry Feeding Allowances, 2005). Commercial feed mixtures contained ground maize, ground wheat and soybean meal, rapeseed oil, mineral ingredients, and DKA Starter (days 1–21) and DKA Grower vitamin premix (days 22–42) (Table 1). The ingredients and nutritive value of these diets are presented in Table 1. The content of these diets in terms of protein, the essential amino acids lysine and methionine, and metabolisable energy meets the requirements for fast-growing broiler chickens (Poultry Feeding Allowances, 2005). Water was provided *ad libitum* through plastic pipes of the water supply system fitted with a regulator and two nipple drinking points in every pen. The experiment was conducted over 42 days.

Housing and management

Broilers were kept in pens, each with an area of 0.76 m^2. Stocking density was 13 birds at the start and around 30 kg live weight per m^2 at the end of the rearing period. Feeder length per bird was 4.25 cm. Pens were littered with wood shavings from deciduous trees. Indoor temperature, relative humidity and air exchange were maintained in accordance with the recommendations for broiler houses with 24 hours of light (Gornowicz et al., 2007). Room temperature was automatically maintained by means of temperature sensors and thermostats, and decreased every 3 to 4 days. Prophylactic vaccination of birds against Gumboro disease was performed at 5 and 12 days of age in drinking water. Chickens were also given Vitazol, a vitamin supplement, dissolved in drinking water (Biowet Drwalew, Poland) at 4-day intervals. Throughout the experiment, feed intake, chicken mortality and feed conversion rate were recorded. The body weight of chickens was determined at 21 and 42 days of age, following a 24-hour feed withdrawal. On day 43 of the experiment, 10 birds (5 males and 5 females) were randomly chosen from each group. Body weight was measured and the birds were stunned and decapitated. Blood samples were collected into heparinised tubes and centrifuged to obtain plasma. Fresh plasma was used to determine glucose level.

The remaining plasma was frozen (–18°C) and stored pending determination of total protein, triglycerides, cholesterol and high-density lipids (HDL). Blood plasma was analysed using Cormay Diagnostic kits measured in a Beckman DU 640 spectrophotometer. Following slaughter and mechanical defeathering, the weight of the hot carcass without head, gizzard, liver, or abdominal fat (fat pads) was recorded. Hot dressing percentage was calculated. Carcasses were hung on racks and held for 24 h in a cold store at 5°C. The next day, chilled carcasses were weighed and cold dressing percentage and chilling loss were calculated. Carcasses were dissected according to the procedure described by Zgłobica and Różycka (1972). Breast and leg muscles, depot fat, skin, and leg bones were separated from half-carcasses and weighed. The weight of individual half-carcass parts was expressed in grams and as a percentage of the whole carcass (Table 2 and 3). Samples of breast muscles were ground and frozen at –18°C pending chemical analyses. After two weeks, the muscle samples were analysed for dry matter, crude protein, crude fat, and crude ash; the samples of thawed plasma were analysed for total protein, triglycerides, total cholesterol and HDL-cholesterol (AOAC, 2007). Blood plasma analyses were performed by the methods described by Kokot and Kokot (2005).

Table 1. Feed composition and nutritive value (g kg^{-1})

Item	Diet	
	Starter, 1–21 days	Grower, 22–42 days
Ingredients		
maize	37.83	33.46
wheat	20.00	27.90
soybean meal	36.00	30.00
rapeseed oil	2.00	4.00
dicalcium phosphate	1.85	1.70
sodium chlorate	0.35	0.35
calcium carbonate	1.10	1.75
L-lysine HCL (78%)	0.08	0.06
Dl-methionine (99%)	0.17	0.16
vitamin-mineral premix[1–2)]	0.50	0.50
mannan oligosaccharide	0.12	0.12
garlic feed additive (ml/kg)[3)]	+	+
Nutrients in 1 kg of dry matter		
crude protein (g)	221.0	218.6
metabolisable energy (MJ)	12.45	12.30
ether extract (g)	6.57	6.09
ash (g)	56.5	6.85
lysine (g)	5.92	10.50
methionine+cystine (g)	10.77	6.66
calcium (g)	8.40	8.30
phosphorus (g)	6.80	6.60

[1)]Supplied to 1 kg of starter diet: vit. A 13,500 IU; vit. D 3,600 IU; vit. E 45 mg; vit. B_1 3.25 mg; vit. B_2 7.5 mg; vit. B_6 5 mg; vit. B_{12} 0.0325 mg; vit. K_3 3 mg; biotin 0.15 mg; nicotinic acid 45 mg; Ca-pantothenate 15 mg; folic acid 1.5 mg; choline chloride 100 mg; Mn 100 mg; Cu 1.75 mg; Fe 76.5 mg; Se 0.275 mg; I 1 mg; Zn 75 mg; Co 0.4 mg; Endox (antioxidant) 125 mg; Sincox (coccidiostat) 1 g and calcium 0.679 g.

[2)] Supplied to 1 kg of grower diet: vit. A 12,000 IU; vit. D 3,250 IU; vit. E 40 mg; vit. B_1 2 mg; vit. B_2 7.25 mg; vit. B_6 4.25 mg; vit. B_{12} 0.03 mg; vit. K_3 2.25 mg; biotin 0.1 mg; nicotinic acid 40 mg; Ca-pantothenate 12 mg; folic acid 1 mg; choline chloride 450 mg; Mn 100 mg; Cu 1.75 mg; Fe 76.5 mg; Se 0.275 mg; I 1 mg; Zn 75 mg; Co 0.4 mg; Endox (antioxidant) 125 mg; Sincox (coccidiostat) 1 g and calcium 0.79 g.

[3)] Garlic feed additive 0.0; 1.0; 1.5–2.25 ml kg^{-1} respectively to control and experimental groups.

Table 2. Performance of Ross 308 broiler chickens fed *ad libitum* complete starter (days 1–21) and grower (days 22–42) diets supplemented with GFA

Item	GFA, ml kg^{-1} of diet				SEM	Sex		P-value		
	control	1.00	1.50	2.25		male	female	garlic	sex	interaction
Body weight (g)										
21 days	698B b	678 Bb	765 Aa	742 Aa	5	749 Aa	693 Bb	0.0000	0.0000	0.0987
42 days	2555 Aa	2575 Aab	2648 ABbc	2711 Bc	13	2806 Aa	2379 Aa	0.0001	0.0000	0.1209
Mortality (% for 42 days)	2.78 Aa	0.10 Cc	0.63 Bb	0.60 Bb	1.42	0.58	0.45	0.0000	0.0952	0.1427
Feed intake (kg/42 days)	4.50 Aa	4.51 Aa	4.68 AaBb	4.85 Bb	0.26	4.70	4.61	0.0001	0.1981	0.0987
Feed Conversion Ratio (kg/kg BWG)	1.79	1.77	1.77	1.80	0.08	1.79	1.77	0.3890	0.2899	0.1516
European Production Efficacy Factor (points)	331 Bb	347 ABab	363 Aa	356 Aa	16	350	348	0.0009	0.3201	0.1999

A, B – values in the rows with different letters differ significantly (P≤0.01).
a, b – values in the rows with different letters differ significantly (P≤0.05).
GFA – garlic feed additive.
SEM – standard error of the mean.
BWG – body weight gain.
DM – dry matter.

Table 3. Slaughter yield, dressing percentage and weight of breast and thigh muscle of Ross 308 chickens fed *ad libitum* complete starter (days 1–21) and grower diets (days 22–42) supplemented with GFA

Item	GFA, ml kg^{-1} of diet				SEM	Sex		P-value		
	control	1.00	1.50	2.25		male	female	garlic	sex	interaction
Slaughter body weight (g)	2522 Aa	2549 ABa	2656 Bb	2643 Bb	38	2806 Aa	2379 Aa	0.0016	0.0000	0.1255
Hot carcass weight (g)	1884 Aa	1915 ABa	1991 BCb	2036 Cb	29	2108 Bb	1806 Aa	0.0007	0.0000	0.1587
Cold carcass weight (g)	1826 Aa	1852 Aa	1924 ABab	1978 Bb	27	2040 Bb	1750 Aa	0.0003	0.0000	0.0993
Chilling losses (%)	3.2	3.4	3.4	2.8	0.2	3.2	3.1	0.4551	0.3498	0.6712
Dressing percentage	72.5 Aa	72.7 Aa	72.4 ABab	74.9 Bb	0.3	72.7	73.6	0.0062	0.1364	0.4713
Absolute weight (g)										
breast muscle	472.8 Aa	491.8 ABab	509.4 ABa	524.4 Bb	7.4	520.4 Bb	478.8 Aa	0.0070	0.0008	0.0494
thigh muscle	391.2 a	399.8 ab	411.4 b	414.6 b	6.8	441.4 Bb	367.0 Aa	0.0470	0.0000	0.9987
gizzard	22.4	23.1	25.2	23.0	0.6	24.6 b	22.3 a	0.2641	0.0357	0.4269
liver	45.7 a	47.7 a	54.1 b	46.9 a	1.3	52.7 Bb	44.5 Aa	0.0291	0.0003	0.3472
abdominal fat	43.1 ABab	38.4 Aa	41.3 ABa	49.6 Bb	1.4	40.9 Aa	45.3 Bb	0.0059	0.0053	0.2155
skin	108.3	110.4	113.2	119.8	2.3	117.3	108.5	0.3109	0.0912	0.4180
leg bones	110.2	106.4	110.2	110.0	1.2	126.7 Bb	93.2 Aa	0.5120	0.0000	0.6268
Relative proportion (% of carcass weight)										
breast muscle[1]	26.0	26.6	26.5	26.6	0.5	25.5 Bb	27.3 Aa	0.7043	0.0004	0.3760
thigh muscle[1]	21.4	21.6	21.4	20.9	0.3	21.6 a	21.0 b	0.4057	0.0370	0.1403
gizzard[2]	1.2	1.3	1.3	1.2	0.0	1.2	1.3	0.3000	0.2218	0.4224
liver[2]	2.5 ab	2.6 ABab	2.8 Bb	2.4 b	0.1	2.6	2.6	0.0039	0.7614	0.0980
abdominal fat[2]	2.4 ab	2.6 ABab	2.2 a	2.5 b	0.1	2.0 Bb	2.6 Aa	0.0050	0.0000	0.4221
skin[1]	6.0	6.0	5.9	6.1	0.1	6.2	5.8	0.9441	0.0655	0.4214
leg bones[1]	5.8	5.6	5.5	5.4	0.1	6.0 Bb	5.2 Aa	0.7481	0.0000	0.5294

For abbreviations see Table 2.

[1] As related to hot carcasses.

[2] As related to cold carcasses.

Statistical analyses

Data obtained in the present study were statistically analysed with ANOVA, using SAS/STAT® v. 5.1 (SAS, 1994-2001) for variation between the mean for the GFA (Tukey's test) and sex (Student's t-test) factors, as well as for interaction between factors. Probability levels of $P \leq 0.05$ and $P \leq 0.01$ were defined as significant differences between treatment means.

Results

Compared to the control group, incorporation of GFA at the levels of 1.00, 1.50 and 2.25 ml kg^{-1} of diet increased the body weight of chickens at 42 days by 1.0, 3.5, and 5.8%, respectively, with a significant difference noted only for the GFA3 group ($P \leq 0.01$, Table 2). Bird mortality decreased significantly, from 2.78% in the control group to 0.10–0.63% in the GFA supplemented groups ($P \leq 0.01$). The average value for feed conversion rate, 1.78, did not significantly differ among the experimental groups. The GFA supplement significantly increased the European Production Efficacy Factor (EPEF) of chickens ($P \leq 0.01$). Compared to the control group, it improved by 4.8, 9.7, and 7.6% for the GFA1, GFA2, and GFA3 groups, respectively. GFA supplementation at the level of 2.25 ml kg^{-1} significantly increased hot and cold carcass weight compared to the control group ($P \leq 0.01$; Table 3). Carcass weight loss after 24-h cooling averaged 3.2% and did not significantly differ among the groups ($P \geq 0.05$). Dressing percentage was significantly higher in chickens from the GFA3 group compared to chickens from the control group ($P \leq 0.01$). As well, breast muscles of the GFA3 group were significantly heavier compared to the control group. The weight of muscles and gizzards did not differ significantly among the groups. The weight of abdominal fat in chickens from the GFA3 group was significantly higher compared to the GFA1 group ($P \leq 0.01$). Chickens receiving 1.50 ml of GFA per kg^{-1} had significantly heavier livers, and those fed 2.25 ml of GFA per kg^{-1} had a greater amount of abdominal fat ($P \leq 0.01$) compared to the other chickens. No significant differences were found in the weight of skin and leg bones ($P \geq 0.01$). Expression of the weight of various carcass parts as a percentage of cold carcass weight showed no significant differences between the groups, with the highest value being liver weight as a percentage of carcass ($P \geq 0.05$). Chickens receiving 1.50 ml of GFA per kg^{-1} diet differed significantly from the other groups in terms of the highest percentage of liver weight in carcass weight ($P \leq 0.01$).

The slaughter weight in males was higher than that of females by 15.2%, with significant differences ($P \leq 0.01$; Table 4). The weight of hot carcass, cold carcass, breast and leg muscles, gizzard and liver, skin and leg bones was significantly higher for males ($P \leq 0.01$). No significant differences were observed in dressing percentage ($P \geq 0.05$). The carcasses of females contained 2.6% abdominal fat compared to 2.0% for males ($P \leq 0.01$). Breast and thigh muscles constituted an average of 47.0% of cold carcass weight, with no significant differences among groups. Along with gizzards and livers, the sum corresponds to an average of 50.8% of edible parts in cold carcasses, 50.0% for males and 52.2% for females.

Table 4. Breast muscle chemical composition of carcass and blood plasma parameters

Item	GFA (ml kg^{-1} of diet)				SEM	Sex		P-value		
	control	1.00	1.50	2.25		male	female	garlic	sex	interaction
Meat chemical composition (% of DM)										
dry matter	25.34	25.35	25.33	25.09	0.09	25.14	25.42	0.6642	0.1033	0.0983
crude protein	22.89 Aa	23.50 ABb	23.68 Bb	23.30 ABab	0.11	23.07 b	23.61 a	0.0089	0.0239	0.3576
crude fat	1.69	1.47	1.32	1.41	0.07	1.60	1.35	0.2141	0.0548	0.2177
crude ash	1.14 Aa	1.16 Aa	1.20 Bb	1.17 ABa	0.01	1.16	1.18	0.0006	0.0628	0.0341
Blood plasma parameters										
glucose (mmol l^{-1})	14.73	14.41	13.92	14.54	0.30	14.80	14.01	0.7801	0.5518	0.7940
total protein (g l^{-1})	38.0 Aa	40.1 ABab	41.4 ABb	43.2 Bb	0.7	40.1	40.6	0.0080	0.7171	0.7283
triglycerides (mmol l$^{-1)}$	0.58	0.52	0.63	0.71	0.03	0.62	0.60	0.3801	0.8839	0.5462
total cholesterol (mmol l^{-1})	3.91	3.93	4.01	4.14	0.07	4.06	3.94	0.7066	0.5181	0.1815
HDL-cholesterol (mmol l^{-1})	2.32	2.41	2.44	2.42	0.04	2.4	2.4	0.5421	0.6106	0.1815

For abbreviations see Table 2.

Adding GFA to the diet of chickens increased the crude protein and crude ash content of breast muscles in all groups, significantly in the GFA2 group ($P \leq 0.01$). A tendency was observed towards lower content of crude fat in breast muscles ($P \geq 0.01$), but this did not result in significant differences in the content of glucose, triglycerides, total cholesterol or high density lipoprotein (HDL) in the blood serum ($P \geq 0.01$). The blood serum total protein content was significantly higher for the GFA3 group compared to the control and other experimental groups ($P \leq 0.01$). There were no significant differences in the content of dry matter, crude fat and ash in the muscles of males and females. No significant interaction of GFA supplementation by sex was demonstrated for the analysed parameters of growth performance, mortality rate, diet intake, feed conversion, carcass parts, or meat and blood plasma composition in broiler chickens.

Discussion

There have been several experiments dealing with garlic dietary supplementation in ruminants (Bampidis et al., 2005; Busqet et al., 2005; Wanapat et al., 2008), pigs (Chen et al., 2008; Lipiński et al., 2011; Grela et al., 2013), poultry (Amouzmehr et al., 2012), and fish (Nya and Austin, 2011), with the objective of determining its effect on the growth and slaughter parameters of animals, quality of products, mortality, diet digestibility, immune resistance, and blood plasma parameters. Garlic was fed to animals in the form of crushed cloves, powder, garlic oil, water and alcohol extracts (Staba et al., 2001). The water extract of crushed garlic bulbs contains allinase and its primary substrates alliin, allicin and allyl sulphides (Staba et al., 2001). They also contain volatile products such as γ-glutamylcysteine and S-allylcysteine. The Allivet additive used in this study is a garlic feed additive with a strong garlic odour, highly soluble in water, and easily mixed with mash. The results of the present study showed that during the starter period (0–21 days), the dietary supplementation at the levels of 1.50 and 2.25 ml kg^{-1} significantly increased body weight compared to the control group. Over the entire rearing period, broilers from the GFA2 and GFA3 groups appeared to attain a heavier body weight compared to the controls. These results are consistent with the previous literature, since dietary supplementation with garlic used in different forms results in a significant increase in body weight. Shi et al. (1999) reported a positive effect of garlic meal on weight gain in broilers, even though other authors (Dey and Samantha, 1993; Javandel et al., 2008; Choi et al., 2010) found no significant effect of garlic dietary supplementation on daily weight gain in broiler chickens. Suriya et al. (2012) investigated the effect of powdered garlic (*Allium sativum*), turmeric (*Curcuma longa*) and cinnamon (*Cinnamonum verum*) on the growth of broilers compared to an unsupplemented group. Specifically, garlic added at 2.5 g kg^{-1} increased the live weight of broiler chickens by 5% and decreased feed conversion rate compared to the other groups. The opposite, i.e. reduced body weight and lower feed conversion rate, were observed when high garlic doses were used. Ari et al. (2012), who fed broilers with diets containing 0 to 20% crushed gar-

lic bulbs, found no significant differences between the groups in body weight, and decreased the plasma cholesterol. In an experiment with Cobb broilers fed 3.0 g kg^{-1} of either garlic or thyme, Amouzmehr et al. (2012) found no significant difference in body weight and feed conversion. It is difficult to compare our findings with those of the previous studies. However, supplements based on garlic extracts appear to be more efficient in stimulating the growth of chickens, as evidenced by the results of Kasuga et al. (2001) and Hoshino et al. (2001). Kasuga et al. (2001) compared raw garlic juice, heated garlic juice, dehydrated garlic powder and garlic extract, and concluded that the pharmacological properties of garlic depend on the way the bulbs are processed. Different bulb extraction methods were also compared in studies reported by Staba et al. (2001).

The positive effect of GFAs on increasing body weight may result from their effect on increasing nutrient digestibility and modulating the microbial composition of gastrointestinal flora, which has been confirmed by several studies. In Cobb chickens, Peinado et al. (2013) found that the substances derived from garlic increased nutrient digestibility and the activity of intestinal mucosa enzymes, and concluded that they may represent an alternative to antibiotics in broiler nutrition. In our study, GFA at the level of 2.25 ml kg^{-1} stimulated the appetite of chickens, which resulted in significantly greater feed intake but did not cause significant differences in the feed conversion rate. Greater feed intake was followed by greater body weight gains. Peinado et al. (2012) investigated the effect of the garlic derivative propyl propane thiosulphonate against broiler enteropathogens *in vivo*. Treatments of 45 to 135 ml/kg had a beneficial effect through reducing the numbers of pathogenic and potentially pathogenic bacteria in the intestine, and also improved the morphological structure of the ileal mucosa and the productive parameters of broiler chickens. An important observation from our study is that over 42 days of rearing, the GFA inclusion reduced chicken mortality to a level of 0.10–0.60%, i.e. between 1 to 6 chickens per thousand. In broiler production, mortality is acceptable up to 4% (40 chickens per thousand). The antibacterial effect of garlic on pathogenic bacteria in the gastrointestinal tract and the activation of the immune system reducing the mortality rate of chickens have been already shown in previous studies. Garlic and garlic products have shown a broad antibiotic spectrum against both gram-positive and gram-negative bacteria (Harris et al., 2001), and have been effective against many common pathogenic intestinal bacteria responsible for diarrhoea in humans and animals (Tatara et al., 2008). Garlic has been extensively studied for its medical properties and has been proven to exert an immunomodulating effect through activating the immune system (Kyo et al., 2001), to reduce low-density lipoprotein levels (Rahman, 2001; Yeh and Liu, 2001), and to exert antioxidant (Borek, 2001) and anticancer activity (Milner, 2001). The immunostimulatory activity of garlic may have contributed to the lower mortality rate of broiler chickens in our study, especially since chicken mortality is highest during the first period of rearing (0–21 days) when the immune system is not fully functional and chickens are exposed to pathogenic bacteria in feed and faeces at a time when the digestive system and the gastrointestinal tract are not fully developed. In a study with Hubbard broilers, Dieumou et al. (2011) demonstrated that garlic extract reduced the number of *Escherichia coli* and *Staphylococcus aureus* in

the small intestine and caecum. They concluded that garlic extract may control pathogens and increase nutrient digestibility in birds. Chang and Chen (2005) showed the stimulating effect of organosulphur compounds from garlic oil on nitric oxide and prostaglandin E_2 in stimulated macrophages, which may be associated with the antibacterial activity of GFA. In a study with Cobb broilers, Peinado et al. (2012) found that a sulphur derivative of garlic is efficient against enteropathogens in broilers. The results of these studies clearly show that garlic addition to the diet at higher levels reduces intestinal disorders in broiler chickens and thus reduces mortality in mass-bred chickens, which improves the economic efficiency of broiler production.

The higher slaughter weight of chickens fed with GFA in our study resulted in higher hot carcass weight, cold carcass weight, and weight of breast muscles, with no differences in the weight of thigh muscles. This is consistent with previous experiments studying the carcass composition of broiler chickens (Brake et al., 1993; Young et al., 2001; Duclos et al., 2007; Połtowicz and Doctor, 2012). Chickens receiving 1.50 ml GFA per kg^{-1} had significantly higher liver weights compared to the other groups of broilers. Increasing the concentration of GFA to a level of 2.25 ml kg^{-1} caused a significant increase in the fat content of chickens' carcasses compared to the control group. This increase may result from higher body weight and higher feed intake, but may also be due to the effect of active garlic substances on the hormonal balance of chickens. This remains a hypothesis and requires further study. The proliferation of adipocytes in chickens is associated with the level of energy feeding during the first weeks of life; their development and growth occurs in the following weeks and results in increased carcass fatness (Tůmová and Teimouri, 2010; Fouad and El-Senousey, 2014). The deposition of abdominal fat results from the accumulation of dietary energy in excess, and also from growth- and age-related hormonal changes (Brake et al., 1993). Abdominal fat in broiler chickens is deposited in the final part of the abdominal cavity as omental and mesenteric fat and fat pads rather than as intramuscular fat. In traditional poultry production, depot fat determined the quality of broth, which is known in Polish cuisine as chicken stock; however, in the technology of portioning poultry carcasses into cuts, depot fat seems to be a redundant product that increases the cost of poultry protein production.

Feeding GFA to chickens increased the crude protein and crude ash content of breast muscles in all the groups, significantly in the group receiving 1.5 ml GFA per kg^{-1}. This suggests a positive effect of garlic on amino acid absorption and balance in the body, although there are no detailed studies in this area. The experimental group in our research showed a tendency towards lower fat content of breast muscles compared to the control group. This is consistent with previous experiments (Bowker and Zhuang, 2013). Lower fat content of breast muscles may negatively affect the flavour and physical parameters of meat such as tenderness and shear force (Zerhdaran et al., 2005). The results reported above are difficult to explain due to the lack of studies investigating the effect of GFAs on the metabolism of amino acids, fats and minerals in broiler chickens and on the composition and properties of broiler meat.

Feeding GFA to chickens significantly increased the plasma level of total proteins, but caused no differences in the content of glucose, triglycerides, total choles-

terol and HDL. In a study with broiler chickens, supplementing garlic at 2% of the diet with or without copper caused a reduction in total cholesterol by 24.2% in red and white meat (Stanaćev et al., 2012). In another study, feeding broiler chickens with diets containing 4 herbs, including garlic at two-day intervals, improved liver function and plasma lipid profile (Manan et al., 2012). In our experiment, the crude protein content of breast muscles was significantly higher in female compared to male muscles, which may be due to differences in hormone metabolism between sexes (Li and Nolan, 2002), although this finding was not confirmed in the literature. No significant differences were found in the dry matter, crude fat and crude ash content of muscles in both sexes, or in the analysed blood plasma parameters. No significant interaction of GFA by sex was found.

Conclusion

It can be stated that the water garlic extract, given continuously in commercial feed as a feed supplement, caused a significant improvement in the body weight of broilers and their parameters while reducing bird mortality during the rearing period. The optimum ration for broiler chickens fed commercial diet containing 210–220 g crude protein kg^{-1} is 1.5–2.25 ml kg^{-1}.

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