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Determination of mechanisms of action of active carbons as a feed additive

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ABSTRACT

Activated carbon's porous structure allows it to adsorb a substrates, products and catalysts from the environment thus modificated the biocatalysis processes in digestive tract. Active carbons are currently used to remove solvents from gas streams and for water purification; however, few studies have examined the mechanisms of action of active carbon during the biotransformation processes in the digestive tracks. The potential benefits of using activated carbon in feed are uncertain because both its chemical and physical properties can vary significantly depending on the type of carbonaceous feedstock. However, the use of active carbons as dietary supplements can also bring many benefits during biotransformation processes in the gastrointestinal tract. Active carbons can adsorb toxins from the gastrointestinal tract and reduce excessive intestinal gas accumulation. The study concerning the adsorption of bacteria and vitamins on the porous structure of various species of active carbons is an important factor to determine their mechanism of action in biocatalysis in digestive system. The use of properly modified activated carbons as feed additives may have a beneficial effect on the development and functioning of breeding animals in the future.

The results of our research show that the active carbon obtained from beech (**KB**), which contained, on average, 14% oxygen content by weight adsorbed bacteria, such as *E. coli* and *S. aureus*, better than all the other active carbons tested. Moreover, the meso- and macropores of carbon seem to contribute little to bacterial adsorption by active carbons. The electron microscopy studies confirmed that the bacteria adhered mainly to the active carbon surface. Our results also indicate that the examined active carbons from beech (**KB**), coconut shells (**TE50**), and hard coal (**RB2**) do not adsorb (or adsorb with very limited efficiency) the vitamins that are routinely added to feed, such as A, B1, D, and K. Broilers fed with feed mixtures supplemented with activated carbon (**KB**) resulted in increases in the weight of the chickens (~2%) after 14 days of application and 2% lower feed consumption (conversion) relative to a control sample. Our data indicate that modifying the surface area and elementary content of active carbon may affect its specificity and selectivity and its capacity to absorb particles used in veterinary, human pharmacy, and cosmetology.

1. Introduction

In recent years, there has been a lot of interest in biochemical reactions and the resulting metabolites due to the activity of the gastrointestinal microbiome; understanding the biochemical pathways of the gastrointestinal microbiota, both in humans and animals, however, is not sufficient to effectively influence the processes that occur in the gastrointestinal tract. The digestive system which is bioreactor of different processes of biocatalysis of farm animals i.a. broilers is becoming a challenge for feed manufacturers and veterinarians, specifically with the withdrawal of antibiotic growth promoters. All disorders associated with the functioning of the digestive tract result in inferior use of the feed and thus decrease the weight of farm animals.

The correct growth of broilers is adversely affected not only by bacterial infections, but also by poor nutrition, which causes abnormalities in digestive system function. Incorrect biotransformation processes in the digestive system result in abnormalities and a lack of vitamins. Also, presence of toxins in the feed can cause very large losses in chicken coops. To prevent the risk of broiler digestive system abnormalities, toxins should be deactivated and the correct digestion should be affected. For this purpose, the breeder should use feed additives and other relevant substances in the poultry feed. Such substances should act on the mucous membrane in such a way that they prevent the growth of pathogenic bacteria [1]. The routine approach is to apply prebiotics as feed additives, but the cheaper way is to use active carbons. Research in this direction is still being developed.

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Charcoal is the carbonaceous residue of wood, cellulose, coconut shells, or other varied industrial waste that has high porosity and high surface area. Activation of charcoal by using chemicals (KOH, acids), carbon dioxide, or water vapour modifies its porous structure. The strongly developed inner surface of active carbon makes it a great absorbent. It can be ground to a very fine powder (> 0.2 mm) and can absorb substances in the amount of nearly 20% of its weight. Moreover, active carbon is nontoxic, which is why this sorbent is applied in the pharmaceutical and cosmetic industries. It is used to remove many toxins from the gastrointestinal tract and the stratum corneum [2–4]. The use of activated carbons increases the catalytic activity of many chemical and biochemical reactions [5]. Its appropriate modification can bring many therapeutic benefits due to its use in biotransformation processes in the gastrointestinal tract.

Adsorption by active carbon depends on its porous structure and the surface area, concentration, and chemical nature of the source of the raw material used for the carbonisation process of active carbon [6]. Active carbons are currently used to remove solvents from gas streams as well as in water purification. Rivera-Utrilla et al. [7] studied the adsorption of *Escherichia coli* on two commercially available active carbons. Active carbon samples have been characterised in terms of their surface area, pore size distribution, elemental analysis, analysis of mineral substances, and pH of the charge zero point. The adsorption capacity of these carbons increased with their hydrophobicity and volume of macropores. The number of bacteria adsorbed on demineralised activate carbon in a solution with a pH equal to the isoelectric point of the coal was negligible [7]. While activate carbon was often used for water treatment, the ability of activate carbon to act as a feed additive and its effect on the biotransformation processes was the subject of few publications.

Oral application of charcoal during *Klebsiella pneumoniae* infection has been reported to reduce the recovery of *Klebsiella* within the colons of mice [8]. In fattening pigs and chicken, active carbon effectively minimised faecal *Salmonella* shedding when used as a feed prebiotic [9,10]; however, Wilson et al. show that the addition of pine charcoal or bamboo charcoal (activated and non-activated) to broiler diets did not alter the recovery of *Salmonella typhimurium* during grow-out [11]. Gerlach and Schmidt found biochar-deactivated toxins that were already in the digestive system and that biochar additionally positively activated intestinal flora and vitality [12]. Doydora et al. and Prasai found that when used as a feed additive for broilers, biochar significantly reduced the amount of ammonia and phosphorus in droppings, therefore requiring a smaller land area on which to spread the litter [13,14]. However, these and other studies also found that too much biochar in the diet can be deleterious. Kana et al. observed depressed growth rates and final body weights for broilers that were fed diets supplemented with 2% or more biochar. It seems obvious that choosing the right amount of carbon is the overriding goal [15].

This study presents the effect of active carbons with appropriate surfaces and the content of elements on the modification of biocatalytic processes in the gastrointestinal tract. The use of active carbons with appropriate chemical and physical properties as dietary supplements for livestock can be an effective way to reduce the use of pharmaceuticals and solve global energy and environmental problems. The results regarding the quality of the analyzed active carbons have allowed to selection of the most effective sample that has been introduced into the broiler feed additives and tested on animals. This work presents the sorption of Gram-positive and Gram-negative bacteria in bacterial culture by four different active carbons: pharmaceutical (**CA**, commercially available), derived from beech (**KB**), derived from coconut shells (**TE50**), and formed from hard coal (**RB2**). Moreover, the vitamin adsorption by activated carbons was tested *in vitro*. The effect of adding active carbon (**KB**) to the feed of rearing broilers is also explored here.

2. Materials and methods

2.1. Characteristics of the porous structure

The active carbons used in this study were derived from beech (**KB**), coconut shells (**TE50**), hard coal (**RB2**) or commercially available (*Aflofarm*, **CA**). For the following studies, a 0.5–3.15 mm sieve fraction was isolated. The following analysis was used in the technical analysis (CHNS EA 1110, CE Instruments): proximate analysis (moisture content according to: ASTM D3173-11), ash content (according to: ASTM D3174–12), volatile matter (according to: ASTM D3175–11), and thermogravimetric studies.

The porous structure of the samples was determined at 25 °C in the thermogravimetric apparatus (TG) using the isotherms of carbon dioxide adsorption in the pressure range of 0–700 mmHg and the adsorption/desorption isotherms of C_6H_6 in the relative pressure range of p/p_o 0–1. For the interpretation of the CO_2 adsorption isotherms, the Dubinin–Radushkevich (DR) theory and equation were used [16]. For the DR equation calculations, the adopted coefficient β affinity was 0.37. Using the DR equation, the micropore volumes available for carbon dioxide compounds were calculated, assuming that each molecule of CO_2 at the temperature of 25 °C is laid flat on the surface of the micropores and covers an area of 0.185 nm^2 [17]. The Brunauer, Emmet, and Teller (BET) theory was used for the interpretation of the benzene adsorption isotherm obtained at the relative pressure p/p_o = 0.01–0.30 [18]. The BET method was used to calculate the material surface (S_{BET}). For the calculations, it was assumed that the surface of the benzene molecule at 25 °C occupies an area of 0.41 nm^2 [19]. Based on the benzene desorption curve in the range of the relative pressure p/po = 0.96–0.175, the mesopores' volume (V_{MES}) and mesopores' size distribution (S_{MES}) as a function of width (2–3; 3–5; 5–10 and 10–50 nm) were calculated. The calculations were made according to the Pierce method [20], taking into account the amendments on the thickness of the adsorbed layer [21]. The calculations assumed that the pores have the shape of a slot. The average mesopore diameter (d_{MES}) was calculated from the following equation: $d_{MES} = 2V_{MES}$: S_{MES}.

The micropore volumes available for benzene vapours (V_{MIK}) were calculated as the difference between the volume of benzene adsorbed at p/p_o = 0.96 and a previously calculated volume of mesopores: $V_{MIK} = V0.96 - V_{MES}$ [$cm^3\,g^{-1}$]. The micropore volumes of the widths below 0.4 nm (submicropores, V_{SUB}) available to smaller molecules (i.e., carbon dioxide) and inaccessible to larger molecules (i.e., benzene) were calculated. Sub-micropore volumes were calculated as the difference between the micropore volume calculated from the CO_2 adsorption curve (V_{MIK} CO_2) and the micropore volume calculated from the benzene adsorption curve (V_{MIK}) at p/p_o = 0.96. $V_{SUB} = V_{MIK}$ $CO_2 - V_{MIK}$.

2.2. Carbonisation and physical activation

Carbonisation was carried out under an argon atmosphere at a flow rate of 10 °C min^{-1}. The carbonisation conditions for the **RB2**, **TE50**, and **KB** samples was the same. The carbonisation process was conducted at up to 600 °C min^{-1} and maintained at this temperature for 60 min. For the T**E50** active carbon, the process of carbonisation was additionally activated by water vapour with heating up to 700 °C up to 50% mass loss of the material (calculated to ash free). For **RB2** active carbon, the activation was carried out by water vapour with heating up to 800 °C up to 50% weight loss of the material.

2.3. Elemental content of active carbons

Scanning electron microscopy (SEM) was used to examine the

elemental content of the active carbons. The samples of active carbon, taken as-is, were observed using a Quanta 250 FEI scanning electron microscope operated at 15 kV. The elemental analysis was performed by means of a Tracor-Northern energy dispersive X-ray (EDX) spectrometer mounted on the Quanta 250 FEI. The EDX detector was equipped with an ultra-thin light-element window to detect elements with atomic numbers > 4. The elemental content results are presented as the mean of content from six independent places on active carbon surface. The results are presented as the percentage of element weight on the active carbon surface ± SD.

2.4. Bacterial sorption

To determine the active carbon's ability to adsorb bacteria, batch biosorption of Gram-positive and Gram-negative representatives was conducted. The samples of activated carbon (**TE50**, **RB2**, **KB**, and pharmaceutical carbon (*Aflofarm*, **CA**)) were conditioned at 120 °C for 15 min to ensure sterility before each experiment. *E. coli* (ATCC 8739) and *Staphylococcus aureus* (ATCC 6538) species were grown in Luria Broth-Miller (LB) and Mueller Hinton (MH) broth, respectively, at the temperature of 37 °C. After 24 h, the culture medium was centrifuged to recover the cells. The sediment was washed three times with sterile deionised water (SDW) and finally the cells were resuspended in 20 ml of SDW. Ten ml of each of the bacteria suspensions were added to each of the two tubes; one contained 0.15 g of active carbon sample and the other was used as a blank. The investigated sample and control sample were stirred by vortexing for 2 min and then shaken at 37 °C (the optimal growth temperature of bacteria culture) with slight agitation (45 rpm). To determine the non-adsorbed, viable bacterial cells, the suspension was serially diluted and plated in agar nutrient broth. To determine the adsorption level, the following equation was used: $A\% = 100\% - ((CFU/ml_{of\ active\ carbon})/(CFU/ml_{of\ control}) \times 100\%)$, where AC is the bacteria solution with active carbon and Control is the control sample (no sorbent attached). The number of bacteria adsorbed per 0.15 g of adsorbent sample was measured as a ratio of the number of non-adsorbed colonies (former units CFU/ml) in samples with carbon to the number of colonies (former units CFU/ml) in the control samples and is expressed as a percentage. All measurements were performed in duplicate. The results are presented as the percentage of adsorbed bacteria ± SEM.

2.5. Electron microscopy studies of bacterial sorption on active carbon

The time taken for broilers to digest feed is between 4 and 8 h. Incubation of active carbon with bacterial cells for 6 h seems to be the most optimal and consistent with the possibility of the adsorption of bacterial cells in the broiler digestive tract. Therefore, after 6 h of contact, the samples of bacteria and active carbon (TE50, RB2, KB, and pharmaceutical carbon CA) were fixed in glutaraldehyde (2.5% in cacodyl chloride buffer). After consolidation, the material was washed three times in cacodilic buffer (pH = 6.8) and then dehydrated with an alcohol series (from 30% to 100% in 10% increments). Dehydrated material was air dried. Dried carbon fragments were glued onto the microscope tables using a carbon strip. The material was dusted with gold (10 nm), and after being polished, the samples were subjected to the Auriga 60 (Zeiss) scanning electron microscope (Zeiss) at a beam speed of 2 kV. Samples were observed using secondary electron detectors (SE2 and InLens).

2.6. Vitamins adsorption

Analysis was carried out by via high-performance liquid chromatography using a Waters 2707 instrument (Waters 1525 Binary HPLC Pump) with a vacuum degasser. A diode array detector (Waters 2489 UV/Visible Detector) was employed for detection. The reverse-phase column SUPELCO 10UM 25 cm × 4.6 mm DISCOVERY was used for separation. HPLC-grade solvents were employed throughout the analysis: acetonitrile (Merck, UK) with 0.05% trifluoroacetic acid and water with 0.05% trifluoroacetic acid. The sample injection volume was 15 µl, the flow rate was 1 ml/min, and the wavelength for detection was 280 and 254 nm. Vitamin B1 (Thiamine Hydrochloride − 99%), vitamin D (Cholecalciferol 98%), vitamin A (Retinyl Acetate 99%), and vitamin E ((+/-)-α-Tocopherol) were purchased from Merck. Each of the vitamins (A, B1, D and E) was dissolved in CH_3CN as a matrix (pH 5–6). For the final concentration of tested vitamins (0.5 mg/ml) the 0.15 g of appropriate sorbent (**TE50**, **RB2**, **KB** and **CA**) was added and mixture was incubated for 6 and 24 h. Analyses were made after incubation time and after removing the sorbent from the matrix. The samples solutions were filtered by Whatmann® filter paper to separate any hard suspended particles (such as carbon). For each of the analysed vitamins, a standard curve for the concentration of the sample from the surface of the signal field was determined. All measurements were performed in triplicate. The results are presented as mean ± SD.

2.7. Experiments on animals

The experiment was carried out on 252 roosters of the Ross 308 variant, from the one-day age. The chicks were divided into 2 experimental groups, each with 14 repetitions, 9 replicates each. The birds were brought up to the 35th day of life in an air-conditioned hen house. Broilers were maintained in a room at a constant temperature of 33 °C in the first week of life, then temperature was lowered weekly by 1.5 °C until reaching 22 °C in the 4th week of life. Humidity of the air was maintained at a level of not less than 55%. In the first three days of life, the light program was 24 h a day, and from the 4th day of life was kept at a constant level of 18 h of light a day. Each playpen was equipped with one feeder and four nipple nipples. Water and feed were available *ad libitum*.

2.8. Preparation of active carbon as feed additives

The birds of all groups were fed the same, free-flowing, complete mixes in the 2-phase system: starter – 1–14 days (AgroMax 1) and finisher – 15–35 days of rearing (AgroMax 3). The mixtures were produced in the Feed Mixture "Agrocentrum" in Kałęczyn in Poland. The broilers were divided on two groups: I control - chickens receiving a standard full-feed; II experimental – chickens receiving the same mixture of feed as control with 0.2% of the selected active carbon **KB** for 0–14 days, and 0.3% of **KB** for 15–35 days. The dose of carbon supplement was selected based on the analysis of the literature [15,22]. The chickens' feed supplemented with active carbon **KB** was mixed by hand three times for 15 min.

2.9. Statistical analysis

Microsoft Excel software was used to analyze the obtained data of means and standard deviations (SD). The results from Table 4 were statistically analyzed with one-way ANOVA followed by Tukey multiple comparisons test, differences with $p < 0.05$ were considered statistically significant using GraphPad Prism software (version 6.0). The results from Table 5 were analyzed statistically using a one-way analysis of variance in the Statistica software for Windows (Statsoft Inc., 2011, version 10). After determining the homogeneity of the variance, the mean values were compared using classical test methods (Duncan's test). The significance level $P \leq 0.05$ was assumed.

3. Results & discussion

Active carbon **KB** contains 4.4% ash, whereas the coefficient $A^{dry\ ash\ free}$ (ash matter of dry ash free) for active carbons **TE50** and **CA** is about ten times lower. Activated carbon **RB2** had the lowest humidity (0.7%) and the highest ash content (5.3%) among the samples tested (Table 1).

Table 1
The characterisation of active carbons **CA**, **TE50**, **KB** and **RB2**.

Name of sample	Proximate analysis		
	Moisture [%]	Ash content [%]	
	$M^{analytical}$	$A^{analytical}$	$A^{dry\ ash\ free}$
CA	5.0	1.7	2.2
TE50	3.1	2.1	2.2
KB	4.2	3.5	4.4
RB2	0.7	5.0	5.3

The lowest volume of micropores and mesopores (0.210 cm^3/g) was observed for active carbon **KB** (Table 2). Analysis of the porous structure indicated that both the **TE50** and **RB2** activated carbons and pharmaceutical carbon **CA**, for the most part, consist of micropores with widths of 0.4–2 nm. However, the area of micropores with widths of 0.4–2 nm is about 43, 49 and 34% lower in the active carbon **KB** than in the active carbons **TE50**, **RB2**, and **CA**, respectively (Table 3). In addition, active carbon **KB** has micropores with volumes < 0.4 nm, whose surface is 24 m^2/g. The mesopore area in pharmaceutical carbon **CA** is much higher than in the other samples tested. The biggest differences were observed for mesopores with widths in the range of 2–50 nm, whose surface area in the **CA** sample is around three times and four times higher than in the **RB2** active carbon and **TE50** and **KB** active carbons, respectively (Table 3).

For these studies of bacterial adsorption, *E. coli* was the representative of Gram-negative bacteria and *S. aureus* was the representative of Gram-positive bacteria. The *E. coli* bacteria showed the lowest degree of adsorption onto the pharmaceutical active carbon **CA** (50%) after 6 h of contact between the cells and the sorbent (Fig. 1A). Additionally, after 24 h of contact, the level of adsorbed *E. coli* cells onto the **CA** decreased to 20%. In the case of active carbon **TE50**, the amount of adsorbed *E. coli* cells remained constant during the 24-hour contact time with the sorbent and fluctuated from 55 to 60% (Fig. 1C). **KB** revealed the highest level of adsorption toward *E. coli* bacteria of 80% after 6 h incubation of bacteria with sorbent. For the active carbon **RB2** the level of bacterial sorption was ~70% after 6 h incubation of bacteria with sorbent. After 24 h, the level of *E. coli* adsorption was higher and it was 100% for **RB2** and 90% for **KB** (Fig. 1B, D).

The tested samples of the active carbons **TE50**, **RB2** and **KB** appear to be better sorption materials for Gram-positive bacteria like *S. aureus*. All the sorbent samples tested (**TE50**, **RB2**, **KB**) showed 80–100% adsorption of *S. aureus* cells, depending on the contact time (Fig. 2B–D). Active carbon **CA** had the worst properties for adsorbing *S. aureus* cells: the bacterial cell adsorption level dropped from 90 to 50% after 6 h of contact (Fig. 2A).

In both cases of Gram-positive and Gram-negative bacteria adsorption *via* active carbons, reductions in the bacterial concentration in water affect the pH of the solution (data not shown). The pH levels of the all tested active carbon samples were determined at the level of 10.3. Variations in solution pH also strongly influence the electrostatic interactions between the active carbon and the adsorbate [9]. Under the pH conditions described above, the pH of the carbon material surface is negatively charged. In lower pH environments, the surface of the material is charged positively. It can be assumed that the active carbon was charged positively when in contact with the *E. coli* and *S. aureus* suspensions (in both cases, the solution pH ranged from 9.3 to 7.9). Along with time, the electrical charge slightly increased. For both bacteria, changes in the solution pH affected the electric charge on the active carbon surface in the same way. For this reason, the pH of the aqueous solution (at the applied range) was not a factor in the significant differences in the efficiency of the adsorption between the Gram-positive and Gram-negative bacteria.

The characteristics of active carbons **TE50**, **RB2** and **KB** and their elemental analysis indicate that the content of oxygen groups is important for the adsorption of *E. coli* bacteria. The highest level of adsorption toward *E. coli* bacteria was found in the **KB** (80%) and **RB2** (70%) active carbons after 6 h of contact between the sorbent and bacteria cells (Fig. 1B, D). **KB** revealed oxygen contents of 14.05% and this level of oxygen is significant different from remaining active carbons **CA**, **TE50** and **RB2** (Table 4). The content of oxygen groups on the surface of **KB** active carbons is approximately two times higher than that of active carbons **CA** and **TE50** (Table 4).

RB2 show a significant different level of metals (such as Na^+, Mg^{2+}, Cu^{2+} and Fe^{2+}) on its surface compare to **CA**, **TE50** and **KB** active carbons. Rivera-Utrilla et al. [7] stated that the amount of adsorbed *E. coli* bacteria on the carbon adsorbent depends on the presence of metal ions in the solution. Metal ions can bind to the surfaces of bacterial cells, mainly by lipopolysaccharides, and to a lesser extent by phospholipids. This increases their hydrophobicity, which positively affects their adsorption on active carbons. The amount of cations bonded to the surface of cells depends on their valence, ionic radius, hydration energy, and hydrate radius. Therefore, in the experiment proposed by Rivera-Utrilla et al. [7], the adsorption from a solution that contained Ca^{2+} cations was greater than that from a solution with Mg^{2+} cations. The metal content on the surfaces of active carbons can have a similar effect toward the adsorption of bacterial cells. However, additional research in this direction should be performed to confirm the nature of this process.

The elemental content analysis of active carbons **RB2** showed that its surface is inhomogeneous (the high SD values; Table 4). However, this does not significantly change the adsorption strength of **RB2** toward bacteria. The high content of oxygen and metals on the surface of the **RB2** sample affects the adsorption properties of the bacteria despite the lack of a homogenous surface of this active carbon.

The level of adsorption of *E. coli* by active carbon **KB** increased over time and was 90% after 24 h (Fig. 1D). This sorbent was also an effective adsorbent of *S. aureus* after a 6-hour contact time (approx. 90% of adsorption, Fig. 2D). However, the remaining active carbons (**TE50**, **RB2**) showed a close (80–90%) level of adsorption after 6 h of contact time with *S. aureus* cells (Fig. 2B, C). The lowest oxygen content was exhibited by **CA** and **TE50** (5.50 and 6.12%, respectively) (Table 4). Only in the case of **CA**-active carbon did we observe a drop of *S. aureus* adsorption from 90 to 50% after 6 h of incubation (Fig. 2A). It is assumed that the content of oxygen groups in the case of Gram-positive

Table 2
The micropore volumes in active carbons **CA**, **TE50**, **KB** and **RB2**.

Name of sample	Micropore volume with widths [nm]:		Mesopore volumes with widths [nm]:					Volume of micropores & mesopores $V_{mic+}V_{mes}$ [cm^3/g]
	< 0.4 $V_{sub\text{-}mic}$ [cm^3/g]	0.4–2 V_{mic} [cm^3/g]	2–3 $V_{2\text{-}3}$ [cm^3/g]	3–5 $V_{3\text{-}5}$ [cm^3/g]	5–10 $V_{5\text{-}10}$ [cm^3/g]	10–50 $V_{10\text{-}50}$ [cm^3/g]	2–50 $V_{2\text{-}50}$ [cm^3/g]	
CA	0.000	0.499	0.051	0.044	0.054	0.056	0.205	0.704
TE50	0.000	0.401	0.016	0.011	0.007	0.101	0.042	0.443
KB	0.009	0.171	0.021	0.005	0.003	0.001	0.030	0.210
RB2	0.000	0.349	0.026	0.011	0.008	0.009	0.054	0.403

Table 3
The distribution of pore surface area in active carbons **CA**, **TE50**, **RB2** and **KB**.

Name of sample	Surface of micropores with widths [nm]		Surface of mesopores with widths [nm]					Surface of micropores & mesopores $S_{mic}+S_{mes}$ [m^2/g]	Surface BET S_{BET} [m^2/g]
	< 0.4 $S_{sub-mic}$ [m^2/g]	0.4–2 S_{mic} [m^2/g]	2–3 S_{2-3} [m^2/g]	3–5 S_{3-5} [m^2/g]	5–10 S_{5-10} [m^2/g]	10–50 S_{10-50} [m^2/g]	2–50 S_{2-50} [m^2/g]		
CA	0.0	1379.0	42.5	23.8	15.8	5.3	87.4	1466.0	1267.0
TE50	0.0	1107.0	13.4	5.8	2.1	0.7	22.0	1129.0	979.0
KB	24.0	472.0	17.6	2.9	0.9	0.1	21.5	518.0	416.0
RB2	0.0	964.0	21.6	6.0	2.5	0.7	30.8	995.0	863.0

bacteria may have a less significant effect due to the lack of lipopolysaccharides on the cell surface, which can form more hydrogen bonds with oxygen atoms.

The obtained results of bacterial adsorption change over time and after 24 h, in the case of **KB** (Fig. 2A–D). Data on the adsorption of bacterial cells from aqueous solution were confirmed by electron microscopy studies (EMS). In addition, EMS studies revealed that the size of the pores is not essential for adsorbing bacteria because they adhere mainly to the surface of the sorbent (Figs. 1 and 2).

These vitamins are commonly added to feed as additives to improve the nutritional value of feed. It is important to maintain proper vitamin control in the feed. Therefore, the ability of active carbons **CA**, **TE50**, **RB2** and **KB** to adsorb vitamins, such as retinyl acetate (vitamin A), thiamine hydrochloride (vitamin B1), (±)-α-tocopherol (vitamin E), and cholecalciferol (vitamin D), was examined after 6 and 24 h of incubation. The active carbon **KB** adsorbed all of vitamins to the lowest degree (Fig. 3A–D). The adsorption of vitamins A, B1, E, and D by pharmaceutical active carbon **CA** was around 100% (Fig. 3A–D). The active carbons **RB2** and **TE50** revealed similar levels of absorption of vitamins A, B1, and E (Fig. 3A–D). **TE50** only showed about a 25% higher level of vitamin D adsorption (60%) after 4 h of incubation than did **RB2** (40%). The obtained results suggest that the size of the pores may play a significant role in vitamin absorption.

The active carbon **KB** has submicropores, which makes the sorption of vitamins more difficult (Fig. 3A–D & Table 2–3). However, active carbons **RB2** and **TE50** have similar pore sizes and their sorption strength with respect to vitamins is similar (Fig. 3A–D & Table 2–3). The area of mesopores with a width of 2–50 nm in active carbon **CA** was the highest relative to the remaining samples and active carbon **CA** had the highest level of sorption of vitamins (Fig. 3A–D & Table 3). Due to the higher level of bacterial adsorption and the low level of vitamin adsorption, active carbon **KB** was used for animal testing to check its properties as a feed additive.

Our preliminary studies indicate that supplementing broiler feed with active carbon **KB** (0.2–0.3%) has a positive effect on broiler mass (Table 5). The use of **KB** a feed additive causes an increase of around 2.5% in the weight of the broilers and thus reduces feed consumption by 2%. In addition, the increase in the weight of broilers indicates that

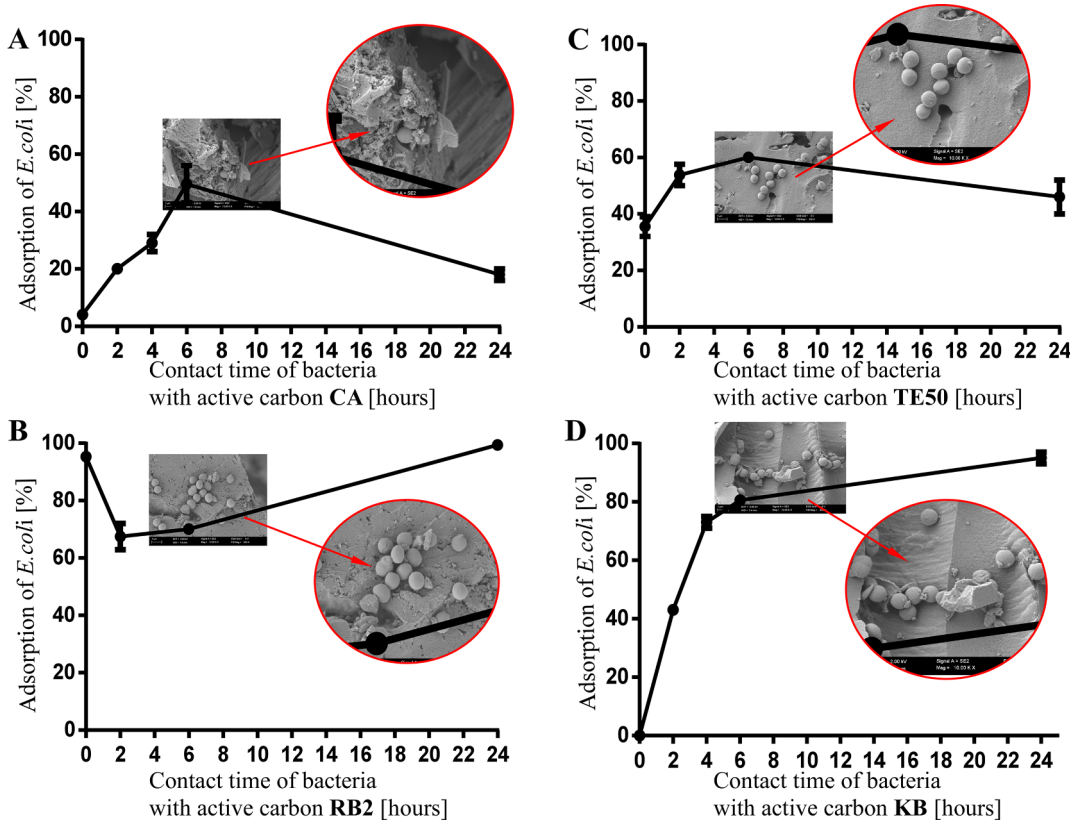

Fig. 1. *Escherichia coli* adsorption on the (A) pharmaceutical active carbon **CA**; active carbon derived from (B) coconut shells **TE50**; (C) coal **RB2**; (D) beech **KB** and electron microscopy studies after 6 h of contact.

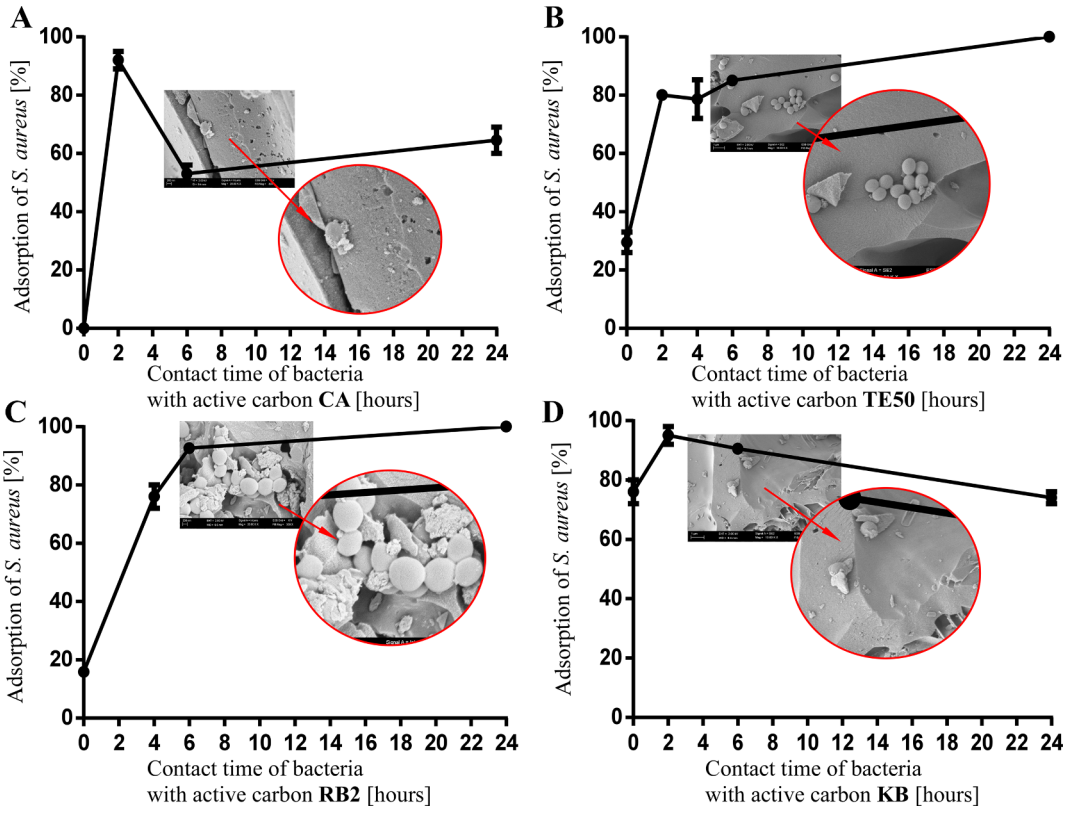

Fig. 2. *Staphylococcus aureus* adsorption on the (A) pharmaceutical active carbon **CA**; active carbon derived from (B) coconut shells **TE50**; (C) coal **RB2**; (D) beech **KB** and electron microscopy studies after 6 h of contact.

Table 4
Elemental content of active carbons **CA**, **TE50**, **RB2** and **KB**.

	Active carbon			
	CA	TE50	RB2	KB
Element	Weight [%]*			
C	93.60 ± 0.74	91.29 ± 0.77	68.78 ± 24.07	84.70 ± 2.55
O	5.50 ± 0.69	6.12 ± 1.51	11.13 ± 13.84	14.05 ± 2.55
Na	0.15 ± 0.05	0.08 ± 0.13	0.78 ± 1.29	–
Mg	–	–	0.27 ± 0.25	0.07 ± 0.08
Cu	–	0.97 ± 0.74	5.92 ± 10.57	–
Al	0.09 ± 0.03	–	0.09 ± 0.13	0.02 ± 0.03
Si	0.06 ± 0.02	0.08 ± 0.08	8.92 ± 12.48	0.05 ± 0.05
P	0.08 ± 0.03	0.03 ± 0.06	–	0.05 ± 0.08
S	0.07 ± 0.03	–	0.25 ± 0.35	0.03 ± 0.06
Cl	0.22 ± 0.15	–	–	–
K	0.09 ± 0.09	1.39 ± 0.48	0.49 ± 0.49	0.33 ± 0.20
Ca	0.13 ± 0.09	–	1.37 ± 1.34	0.70 ± 0.75
Rn	–	0.05 ± 0.11	–	–
Fe	–	–	1.99 ± 3.71	–

The elemental content results are presented as the mean of content from independent places on active carbon surface.

* The results are presented as the percentage of element weight on active carbon surface ± SD.

the natural bacterial flora of chickens is not disturbed by the use of activated carbon **KB** as a supplement. Our research will continue towards identifying the sorption properties of natural broiler microflora by activated carbons.

4. Conclusions

The active carbon **KB** derived from beech was selected as the most effective sample for application as the feed additive for broilers. **KB** revealed the high level of Gram-positive and Gram-negative bacterial adsorption (90 and 80%, respectively) after 6 h contact of bacteria with sorbent. Moreover, **KB** active carbon adsorb with very limited efficiency the vitamins that are routinely added to feed, such as A, B1, D, and K. The addition of active carbon **KB** to feed improved the performance of broiler chickens, including an approximately 2.5% increase in their body weight and an approximately 2.0% better feed conversion ratio.

Studies on the use of pharmaceutical active carbons (**CA**) and active carbons from coconut shells (**TE50**), hard coal (**RB2**), and beech (**KB**) as bacterial sorbents resulted in the adsorption of *E. coli* at the levels of 50%, 60%, 75%, and 80% (6 h of contact cells with sorbent), respectively. The adsorption of *S. aureus* was 50%, 80%, 90%, and 90% (6 h of contact cells with sorbent) for **CA**, **TE50**, **RB2** and **KB**, respectively. Moreover, the meso- and macropores of the active carbons do not seem to affect their bacterial adsorption. In contrast, in the case of vitamin sorption, the surface and volume of the pores do influence the active carbons' adsorption. Elemental content and EMS analysis of active carbons **CA**, **TE50**, **RB2** and **KB** indicate that the oxygen content is important in the adsorption of Gram-positive and Gram-negative bacteria, such as *S. aureus* and *E. coli*, respectively. The higher the content of oxygen groups on the surface of the carbon, the better the adsorption of bacterial cells.

Although the use of active carbons in pharmacy has been known for a long time, the presented data shed new light on the mechanism of action of active carbons. Through appropriate modifications of sorption surfaces, an effective and selective product can be obtained for the sorption of specific bacteria. Specific and selective sorption of activated carbon can have a positive effect on biocatalysis reactions in the gastrointestinal tract. An additional benefit of using active carbon as a diet additive is the reduction of odours and the nuisance of environmental processes. Because active carbon can fulfil two sorbent and catalyst functions in biochemical reactions in livestock, further research in this area will continue.

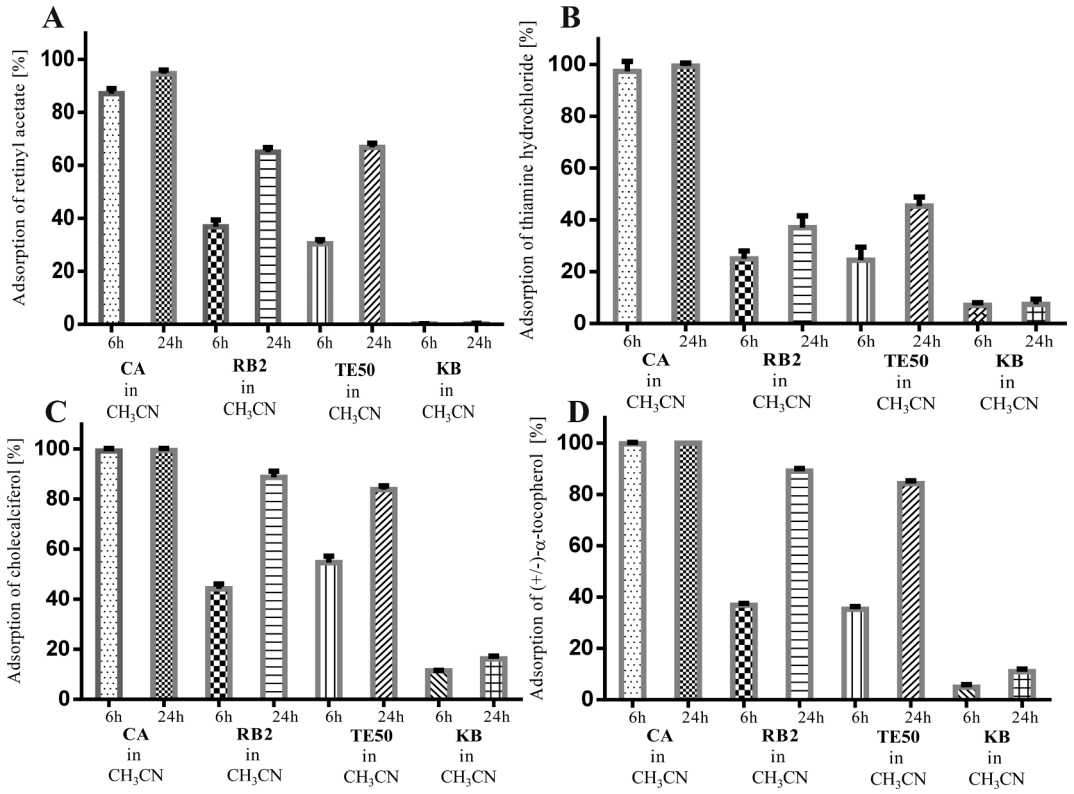

Fig. 3. Adsorption of vitamins (A) retinyl acetate (Vit. A); (B) thiamine hydrochloride (Vit. B1); (C) cholecalciferol (Vit. D); (D) ± -α-tocopherol (Vit. E) by **CA**, **RB2**, **TE50** and **KB** after 6 and 24 h of incubation. All measurements were performed in triplicate. The results are presented as mean ± SD (error bars). The 100% of vitamin adsorption means that concentration of vitamin in CH_3CN after incubation time with sorbent and after filtration process was 0 mg/ml. No vitamin adsorption means that the concentration of vitamin is 0.5 mg/ml in the solvent after incubation time with sorbent and after filtration process.

Table 5
The effect of active carbon **KB** as a feed additive on broiler breeding results. Broiler chickens were given feed with 0.2% activated carbon for days 0–14 and 0.3% activated carbon for days 15–35.

Description	Group	
	I – Control	II –Experimental (active carbon **KB**)
Body weight, kg		
14 days	0.393 ± 0.021	0.402 ± 0.014
35 days	2.030 ± 0.079	2.043 ± 0.100
Feed consumption, kg/kg		
14 days	1.410 ± 0.094	1.382 ± 0.072
35 days	1.454 ± 0.032	1.448 ± 0.036
Mortality, % (1–35 days)	0.79	0.79

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Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.bioorg.2019.02.029.

References

[1] M.E. Abd El-Hack, D.H. Samak, A.E. Noreldin, K. El-Naggar, M. Abdo, Probiotics and plant-derived compounds as eco-friendly agents to inhibit microbial toxins in poultry feed: a comprehensive review, Environ. Sci. Pollut. Res. Int. 25 (32) (2018) 31971–31986, https://doi.org/10.1007/s11356-018-3197-2.
[2] R.R. Dalvi, A.A. Ademoyero, Toxic effects of aflatoxin B 1 in chickens given feed contaminated with aspergillus flavus and reduction of the toxicity by activated charcoal and some chemical agents, Avian Dis. 28 (1984) 61–69, https://doi.org/10.2307/1590128.
[3] R.R. Dalvi, C. McGowan, Experimental induction of chronic aflatoxicosis in chickens by purified aflatoxin B1 and its reversal by activated charcoal, phenobarbital, and reduced glutathione, Poult. Sci. 63 (3) (1984) 485–491, https://doi.org/10.3382/ps.0630485.
[4] D.C. Sands, J.L. McIntyre, G.S. Walton, Use of activated charcoal for the removal of patulin from cider, Appl. Environ. Microbiol. 32 (3) (1976) 388–391.
[5] B. Narowska, M. Kułażyński, M. Łukaszewicz, E. Burchacka, Use of activated carbons as catalyst supports for biodiesel production, Renew. Energy 135 (2019) 176–185, https://doi.org/10.1016/j.renene.2018.11.006.
[6] H. Marsh, F. Rodriguez-reinoso, Activated carbon, Technology 94 (2006) 536, https://doi.org/10.1016/0160-9327(81)90123-X.
[7] J. Rivera-Utrilla, I. Bautista-Toledo, M.A. Ferro-Garca, C. Moreno-Castilla, Activated carbon surface modifications by adsorption of bacteria and their effect on aqueous lead adsorption, J. Chem. Technol. Biotechnol. 76 (2001) 1209–1215, https://doi.org/10.1002/jctb.506.
[8] N. Grall, L. Massias, T.T. Nguyen, S. Sayah-Jeanne, N. Ducrot, E. Chachaty, J. De Gunzburg, A. Andremont, Oral DAV131, a charcoal-based adsorbent, inhibits intestinal colonization by β-lactam-resistant Klebsiella pneumoniae in cefotaxime-treated mice, Antimicrob. Agents Chemother. 57 (11) (2013) 5423–15245, https://doi.org/10.1128/AAC.00039-13.
[9] G.M. Chu, C.K. Jung, H.Y. Kim, J.H. Ha, J.H. Kim, M.S. Jung, S.J. Lee, Y. Song, R.I.H. Ibrahim, J.H. Cho, S.S. Lee, Y.M. Song, Effects of bamboo charcoal and bamboo vinegar as antibiotic alternatives on growth performance, immune responses and fecal microflora population in fattening pigs, Anim. Sci. J. 83 (2) (2013) 113–120, https://doi.org/10.1111/j.1740-0929.2012.01045.x.
[10] S. Watarai, Tana, Eliminating the carriage of Salmonella enterica serovar enteritidis in domestic fowls by feeding activated charcoal from bark containing wood vinegar liquid (Nekka-Rich), Poult. Sci. 54 (4) (2005) 515–521, https://doi.org/10.1093/ps/84.4.515.
[11] K.M. Wilson, D.V. Bourassa, A.J. Davis, M.E. Freeman, R.J. Buhr, The addition of charcoals to broiler diets did not alter the recovery of Salmonella Typhimurium during grow-out, Poult. Sci. 95 (3) (2016) 694–704, https://doi.org/10.3382/ps/pev371.
[12] H. Gerlach, H.P. Schmidt, Biochar in poultry farming, Ithaka J. 1 (2012) 262–264.
[13] S.A. Doydora, M.L. Cabrera, K.C. Das, J.W. Gaskin, L.S. Sonon, W.P. Miller, Release of nitrogen and phosphorus from poultry litter amended with acidified biochar, Int.

J. Environ. Res. Public Health 8 (5) (2011) 1491–1502, https://doi.org/10.3390/ijerph8051491.
[14] T.P. Prasai, K.B. Walsh, S.P. Bhattarai, D.J. Midmore, T.H. Van Thi, R.J. Moore, D. Stanley, Biochar, Bentonite and zeolite supplemented feeding of layer chickens alters intestinal microbiota and reduces campylobacter load, PLoS One 11 (4) (2016) e0154061, , https://doi.org/10.1371/journal.pone.0154061.
[15] J.R. Kana, A. Teguia, B.M. Mungfu, J. Tchoumboue, Growth performance and carcass characteristics of broiler chickens fed diets supplemented with graded levels of charcoal from maize cob or seed of Canarium schweinfurthii Engl, Trop. Anim. Health Prod. 43 (1) (2011) 51–56, https://doi.org/10.1007/s11250-010-9653-8.
[16] L.V. Dubinin, M.M. Radushkevich, The equation of the characteristic curve of activated charcoal, Proc. Acad. Sci. Phys. Chem. Sect. 55 331 (1947), https://doi.org/10.4236/ojs.2014.41001.
[17] Y. Toda, M. Hatami, Y. Toyoda, Y. Yoshida, H. Honda, Micropore structure of coal, Fuel 50 (1971) 187–192, https://doi.org/10.1016/0016-2361(71)90008-1.
[18] S. Brunauer, P.H. Emmett, E. Teller, Adsorption of gases in multimolecular layers, J. Am. Chem. Soc. 60 (2) (1938) 309–319, https://doi.org/10.1021/ja01269a023.
[19] T. Siemieniewska, A. Albiniak, E. Broniek, J. Kaczmarczyk, A. Jankowska, B. McEnaney, X.S. Chen, E. Alain, G. Furdin, D. Bégin, Porosity development in steam activated chars from mixtures of coal tar pitch with graphite-FeCl3intercalation compounds, Fuel 77 (6) (1998) 509–517, https://doi.org/10.1016/S0016-2361(97)00243-3.
[20] C. Pierce, Computation of pore sizes from physical adsorption data, J. Phys. Chem. 57 (2) (1953) 149–152, https://doi.org/10.1021/j150503a005.
[21] S.J. Gregg, K.S.W. Sing, H.W. Salzberg, Adsorption surface area and porosity, J. Electrochem. Soc. 114 (11) (1967) 279C, https://doi.org/10.1149/1.2426447.
[22] H.R. Kutlu, I. Ünsal, M. Görgülü, Effects of providing dietary wood (oak) charcoal to broiler chicks and laying hens, Anim. Feed Sci. Technol. 90 (3) (2001) 213–226, https://doi.org/10.1016/S0377-8401(01)00205-X.