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INVESTIGATION OF THE EFFECTS OF COCONUT, PALM OIL AND SUNFLOWER OIL SUPPLEMENTATION ON FEEDING, PRODUCTION AND SOME MEAT QUALITY, BIOCHEMICAL AND HISTOLOGICAL PARAMETERS OF BROILER CHICKENS

DOCTORAL DISSERTATION THESIS

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1. INTRODUCTION

Today, the expectations and challenges agriculture facing with are dynamically changing. We know that development and large quantities of food production are essential. That is why researchers and breeders are under the same pressure as animal farmers. In recent years, the coronavirus epidemic, the shutdown of the HORECA sector (hospitality industry) and the periodically recurring bird flu outbreaks have sensitively affected the participants of the poultry sector, which was further aggravated by the energy crisis that started in 2022 and the drastic increase in feed prices. In December 2020, Hungary's poultry population amounted to 35.379 million individuals, which further declined to 34.984 million individuals in December 2022 due to bird flu outbreak. This figure not only represents huge drop compared to the stock of 39.208 million in 2019, but also falls significantly short of the 37.016 million in 2000 (KSH, 2023a).

As a result of the breeding development, performance of the different breeds and hybrids were modified in such a way that better feed efficiency and higher meat production could be achieved in shorter time and with less energy consumption on the farms. Due to this genetic change, it was also necessary to change the management and feeding technology. However, high production and good yields can even come at the expense of quality. This may endanger the safe and continuous supply on the market, regardless the quantity produced. Poultry are essential for healthy human diet; their products have high nutritional value and are highly favourable for a wide range of consumers. In the Western World, with the rise in living standards, a group of consumers has emerged that takes product safety and quality as a primary consideration in its customer decisions and has the appropriate spending power. This presents another challenge to the sector.

In recent years, changes in the domestic poultry meat consumption have been observed that are exactly the opposite of the trends in production. Thus, the annual consumption of poultry meat has been increasing continuously, so by 2019 it has reached already the outstanding 35.7 kg/person/year, which significantly exceeds average consumption in the EU. However, in 2021, consumption went down somewhat to 33.3 kg/person/year (KSH, 2023b).

For the sake of healthy nutrition, it is increasingly widely expected that food of animal origin does not contain residues of antibiotics and antibiotic growth promoters. As a result, antibiotic-free feeding regime was started in poultry farming according to the national decree of 128/2009 in our country. (X. 6.) In accordance with the provisions of the national decree, currently antibiotics can only be given to poultry under veterinary prescription and exclusively for therapeutic purposes, and the use must be

strictly documented. Therefore, the emphasis is currently placed on the prevention of diseases in animal husbandry.

Most pathogenic microorganisms can multiply in a slightly acidic, near-neutral environment. Therefore, any procedure results in shifting the pH in the large intestine towards the acidic range (pH<4.5) helps to suppress and reduce the growth of pathogens. Certain fatty acids found naturally in feed or added as supplements can have such effect. Short-chain fatty acids (SCFA) (propionic acid, acetic acid, butyric acid), for example, are known to have significant antimicrobial effect. At the same time, due to their volatility, these fatty acids are not stable when mixed into feed, so their quantity is not constant. On the other hand, medium-long carbon chain fatty acids (MCFA) are not volatile, do not dissociate in the intestinal tract, and according to the literature, they can be used effectively to alleviate the symptoms of necrotic enteritis caused by *Clostridium perfringens* and *Escherichia coli*, which is attributed to their bacteriostatic effect (Jansman *et al.*, 2006; Jang *et al.*, 2007). In this regard, caprylic acid is proven to be the most effective (Skrivanova *et al.*, 2006; Lee *et al.*, 2015). Among the fat sources used primarily for energy replacement in the feed industry, coconut and palm oils are rich in medium-chain fatty acids (primarily lauric acid (C12) and myristic acid (C14)). Thus, an appropriate mixture of them might help to reduce the growth of the aforementioned pathogens, and consequently might enhance immune response to subclinical infections, and prevent performance decline (Dayrit, 2015; Jansman *et al.*, 2006; Nitbani *et al.*, 2016; Skrivanova *et al.*, 2006).

1.1. Objectives

Purpose of my research was to investigate the effect of fat and oil supplements on performance of broiler chicken. Furthermore, since the additional fat can lead to increase in the lipid content of meat cuts, it was also aimed to study whether the used doses of coconut and palm oil could have any effect on fat content and fatty acid composition of chicken meat. Also blood parameters characterizing fat metabolism/ (cholesterol and triglyceride concentration) and antioxidant defence parameters were measured.

Besides the lipid composition, also the sensory quality of meat was studied to define whether the fat supplementation had any influence on the shelf life or the consumer perception of the raw meat.

My objectives also included searching for morphological changes in the digestive tract (e.g. liver, intestine, etc.) and for signs of bacteriostatic effect of the supplemented coconut and/or palm oil. As part of the latter investigations, presence of *Salmonella spp.*, *Clostridium perfringens*,

Escherichia coli, as well as the total microbial counts were analysed in the excreta

2. MATERIAL AND METHODS

There were 240 Cobb 500 hybrid roosters included in the study. The fat and oil supplements used in the experiment were included in the diet from the 21st to the 42nd day of life. There were one control, and four treatment groups created in the experiment, in the latter ones certain oil supplementations were used as it follows: 5% coconut (KO) or palm oil (PO), or sunflower oil (NO) (as positive control). In the last treatment group mixture of 2.5% coconut and 2.5% palm oil mixture was added in the diet (KOPO).

Compound feed of the different groups was analysed for their crude nutrients (protein, fat, fiber, ash) together with starch and sugar content. Also, the fatty acid composition of the fat sources and that of the compound feedswere also measured.

Production parameters were recorded on days 21, 28, 35 and 42 of life (i.e. on days 0, 7, 14, 21 of treatment). Mortality was recorded daily, body weight and feed consumption were measured on weekly basis, and based on these data daily weight gain and feed conversion ratio were calculated. Also, litter samples with excreta were collected for microbiological test at the start of the treatment and then on weekly basis.

Each week of the experimental period exterminations were done, and tissue samples were taken. Weight of the liver, spleen, heart, *bursa Fabricii*, and the right and left breast fillet were measured, and relative organ weights were calculated referred to the grill weight.

At each extermination blood samples were taken from the jugular vein complex (*aa. carotis ext. et int., v. jugularis*). The sampling tubes contained anticoagulant 0.2 mol/L EDTA-Na_2 at 0.05 ml/ml blood. Blood plasma was separated by centrifugation at 2500 rpm for 15 minutes. Then red blood cells (RBC) were washed with physiological saline (0.65% w/v NaCl) and then haemolyzed with adding double-distilled water at a ratio of 1:9 (v/v). These samples were stored at -18°C and -72°C until analyses.

Physicochemical parameters of meat were analysed in the breast fillet. Measuring pH was performed 45 minutes and 24 hours after slaughter. After 24 hours cooling, colour of the meat was analysed (in the Lab colour system) with reflectance spectrometry. For drip loss analysis fresh meat samples were used, while kitchen losses and shear force values were measured after frozen storage.

Biochemical parameters were measured in blood (plasma and RBC haemolysate) samples and in liver homogenates. These latter samples were prepared with a 1:9 ratio of cold (+4°C) 0.65% (w/v) physiological saline solution, using an Ultra Thurrax homogenizer (8000 rpm). The malonyl dialdehyde (MDA) concentration was measured in the native homogenate, while the glutathione peroxidase (GSHPx) activity, reduced glutathione

(GSH) concentration, and protein content were measured in the supernatant fraction of the homogenate (10,000 rpm, +4°C) (Mézes, 1999). Same parameters were also measured in the blood plasma and RBC haemolysates, while in the blood plasma also cholesterol and triglycerol concentration were analysed.

Histological sections from the *bursa Fabricii*, the *Thymus*, the liver and the ileum were prepared at exterminations. After fixing and storing the samples in a 10% buffered formaldehyde solution, they were dehydrated and embedded. The sections were made with a Reichert-type sled microtome (with the help of Renáta Pop, Department of Pathology, University of Veterinary Medicine). Then hematoxylin-eosin staining was applied. The microscopic images were digitalised with using Panoramic MIDI II section scanner and analysed with 3DHISTECH CaseViewer (2.4.0.119028) software.

The microbiological examination of the stool samples was carried out by an independent laboratory. Microbiological analyses methods were as follows: MSZ EN ISO 6579:2002/A1:2007for *Salmonella spp.* , MSZ ISO 16649-2:2005 for *Escherichia coli*, MSZ EN ISO 7937:2005 for *Clostridium perfringens*, MSZ EN ISO 4833-1:2014 for total germ counts.

Statistical analyses of data were performed in R 3.4.4. and R 3.6.1. program, while one-way ANOVA was used to compare the results of the control and experimental groups. As a post-test, Pearson correlation analysis was done with significance level of $p \leq 0.05$. Data distribution was tested with the Shapiro-Wilk test and Q-Q diagram. When significant difference was found ANOVA post-test, the Tukey test was performed.

Based on Guilford (1950), classification of the correlation coefficients into categories according to strength was done.

3. RESULTS AND DISCUSSIONS

3.1. Results of feed tests

The apparent metabolizable energy content (AME) of the complete feeds exceeded the control value with 0.5-1.0 MJ/kg in each experimental group. As a result of fat supplementation, the amount of crude protein decreased proportionally. At the same time, the nutrient content and composition of the four experimental feeds containing different fat supplements did not differ significantly from each other.

According to the results of the fatty acid composition test the three fat sources were significantly different. In alignment with literature data, coconut oil was found to contain high rate of lauric acid, while palm oil was rich in palmitic acid and sunflower oil had high linoleic acid content.

The proportion of lauric acid in the KO feed containing 5% coconut oil increased with 29.27% compared to the control (K), and in the KOPO containing the combined (2.5% coconut oil + 2.5% palm oil) supplementation showed 14.38% increase.

The proportion of palmitic acid has also changed as a result of the supplementation of palm oil, i.e. with the addition of 5% palm oil (PO) 19.01% increase was found, while in Group KOPO the increase was 8.69% compared to the control (K).

The proportion of linoleic acid in feed K was relatively high as thethe commercial broiler chicken diet used as control feed has already contained some sunflower oil to ensure optimal energy content. In the experiment, an additional 5% sunflower oil was added to this feed in the NO group, which only resulted in a 6.59% increase in the proportion of linoleic acid.

3.2. Production parameters

During the experiment, there were 3 deaths altogether in Group K and 2 deaths in Group KO. Thus, the viability was 93.75% in the control and 95.83% in the KO groups. There were no deaths in the other experimental groups. The sporadic deaths experienced cannot be related to the treatment and are within the technological tolerance.

In terms of feed consumption, in the first week of feeding the experimental diets, the feed intake was the highest the PO group. Between days 28 and 35 the trend was similar, but the extent of the deviations has changed. The feed consumption in Group KO and NO was lower than in Group K, while in Group PO and KOPO it was still higher than that. In the final week, all experimental groups fell behind the K group considering feed intake.

Considering weight data, Group KOPO showed the highest value on the 21st and 28th days of life. At 35 days of age, this advantage was true only against the three experimental groups. while the control birds had the

highest weight. On the 42nd day of life, the Groups K, PO, KO ended the experiment with almost the same body weight, and the birds in Group NO were somewhat smaller During the entire experiment, the body weight of the NO group was the lowest.

In the first week of the treatment period (between days 21 and 28) the calculated average daily weight gain was almost the same in each group. Same was true for the feed conversion ratio (FCR). Later, on the 5th and 6th weeks of life feed efficiency has been improved and this change was more pronounced in the treatment groups, than in the control (K). However, it is important to note that for feed consumption and FCR it was not possible to perform statistical analysis due to group feeding, while considering other production parameters, the differences between the groups were not statistically significant.

In terms of breast yield, Group K showed better results compared to the experimental groups during the entire experiment, and on day 28, the difference was statistically significant between K and NO.

3.3. Results of meat quality parameters

The pH values measured 45 minutes after slaughter was higher than the values measured after 24 hours cooling. This corresponds to the standard pH value (5.8-6.0) (Dransfiel and Sosnicki, 1999, Fletcher, 1999, Brewer *et al.*, 2012). At the second extermination, 45 min after slaughter the pH values in Group KOPO, NO and PO were significantly more acidic compared to the control. However, after the 24-hours cooling period, marked differences were found only among the treatment groups, thus pH in the KO and PO groups was significantly different than in the NO group, and even in the samples of the combined treatment (KOPO) it was significantly more acidic compared to the KO group. At the third extermination, 45 min after slaughter, only the difference between Group K and PO was statistically verifiable. After 24 hours cooling, the values were almost the same in Group K, KO and PO, while in Group NO, the pH was significantly less acidic than in the three other treatments.

Considering meat colour, the L* (lightness), a* (redness) and b* (yellowness) values were measured on a fresh cross section surface, and no differences were found among the groups in the three parameters one by one.

However, when the colour difference, or ΔE*ab, was evaluated with the combined analysis of the three parameters according to Lukacs et al., 1982, Group NO meat colour was found to be visually different from the others.

The water-holding capacity of meat was determined with measuring the loss of moisture content by gravity. Significant difference was found

only at the 2nd sampling, when the loss was the highest in the PO group, which was statistically different from the value measured in the group KO. When the tendencies of drip loss are considered over the whole experimental period, it can be concluded that the water-holding capacity of the meat increased continuously.

Considering kitchen processing losses (thawing, frying, cooling), at the time of the first sampling, the greatest loss occurred during cooling, regardless of treatment, while it was the lowest during frying. Otherwise, the losses were hectic at different samplings and in different technological steps of processing. When the overall loss is analysed, the lowest value was found in the control group at the first sampling (although no significant difference among the groups was detected). On day 35, the loss found in group KO was significantly lower compared to the other experimental groups. On day 42, the loss was higher in each treatment groups than in the control, but the difference was statistically verifiable only for NO. Another observation is that the total loss at the end of the experiment was reduced in each group compared to the values measured at the first sampling. This result is consistent with the trends observed for drip loss, indicating that the water-holding capacity of meat improves with age. It can also be concluded that the treatments used in my experiment clearly have an adverse effect on the water-holding capacity of the meat at the normal slaughter time (42 days of age), since the lowest loss was measured in the control group during both cooled storage and processing.

Shear force values measured at first sampling were low, which is in accordance with the young age. At the second slaughter, the value of group K remained similar, while in the other groups it has increased, especially in group KOPO, where the average shear force value was 30% higher than earlier. It was also revealed that the texture of the meat in groups KO and PO was considerably softer than that of the control, and even that of the KOPO meat. In the samples from the third slaughter, shear force values were the best in group PO, and the advantage compared to the groups K and KOPO was statistically verifiable.

Considering nutrient content of the breast meat, three parameters were measured - moisture, protein and fat content. According to the results of moisture analysis, difference was found only at the second sampling (35 days of age), when samples of the group NO contained almost 1.5% more water than the others. The protein content was variable even at the first sampling (day 28). Thus, it was lower in the meat of the groups KOPO and NO than in the others, and the difference was significant between. groups KOPO vs. KO, and vs. PO. By the 35th day of life, the difference continued to increase to such an extent that the group NO breast fillet contained significantly less protein compared to the values measured in groups K, KO,

and PO. However, by the time of normal slaughter (42 days of age), the differences were more-or less vanished. Although the protein content measured in the samples of the NO group was still somewhat lower compared to most of the other groups, the difference could no longer be statistically verified. However, this time the lowest protein content was measured in the meat of the group KO. Regarding fat content, similar patterns were found at the different samplings, and statistically verifiable differences were detected only on day 28, when the sunflower oil (NO) and combined fat supplementation (KOPO) caused reduced meat lipid content, and the difference was significant compared to the samples of K and KO.

The fatty acid composition of breast meat was analysed by an independent laboratory in samples from the slaughters at day 35 and 42. The results from the two samplings show the same pattern. Lauric acid was detectable in the KO and KOPO samples, and its proportion was in line with the inclusion rate of the coconut oil. In groups PO and KOPO, although dose dependence was less evident, increased proportion of palmitic acid was observed. While in group NO, the proportion of linoleic acid was increased significantly compared to the control. These results support the assumption that fat supplementation affects the fatty acid composition of meat.

3.4. Biochemical parameters

Examining the parameters suitable for characterizing fat metabolism, blood plasma cholesterol concentration was elevated on day 42 compared to the values of the former samplings, while, surprisingly, the triglycerol concentration did not even reach the initial values. Also, no significant difference was found between the treatments in the latter parameter, while the cholesterol concentration rose beyond the control value in each experimental group, however, the difference was statistically verified only against KO.

The blood plasma protein content was the lowest in the KOPO group and the highest in the KO birds. With the exception of NO, the latter group values were significantly different from both the control and the other treatments regarding this parameter. Considering the antioxidant defence system, the pattern of glutathione peroxidase activity was the opposite in the groups. Thus, the lowest activity was measured due to coconut oil supplementation, and the difference was significant compared to the values of the control and the other treated groups. The highest enzyme activity was detected in the combined treatment (KOPO), which was significantly exceeded the value of NO. Plasma GSH concentration shows exactly the same pattern. The malonyl dialdehyde concentration was similar in each group. On day 42, GSHPx activity and GSH concentration have increased

in each group compared to the values on day 35, but the differences between the groups remained similar. Thus, at third sampling, KOPO values were the highest for both parameters, and the difference was significant compared to the PO group.

In the red blood cell hemolysate the antioxidant data were typically similar in each group at each sampling. The only significant difference was detected on day 28, in the glutathione peroxidase activity, when coconut oil supplementation resulted in significant increase compared to the control, PO and KOPO groups. Similar pattern was revealed for the reduced glutathione concentration however, the differences were not statistically confirmed. At the same time, malonyl dialdehyde concentration developed similarly in all groups during the entire experimental period.

In the liver samples, in addition to the glutathione redox parameters, the concentration of conjugated dienes and trienes referring to lipid peroxidation induction were also measured. However, no significant difference was observable among and within the samplings. Most of the analysed parameters developed similarly during the entire duration of the study, regardless of the treatments. However, the malonyl dialdehyde concentration gradually increased somewhat in each group as the birds grew older.

3.5. Histological studies

As part of the histological analysis liver samples were studied. The most frequent abnormality was the lipid infiltration (vacuoles with intact edges) and it was present dominantly, when coconut oil or palm oil was supplemented in the diet. Besides, some sporadic signs of local inflammation were identified, which occurred as lymphocyte invasion (primarily in the portal regions) in all examined samples.

No malformations were found in the ileum samples. All the tissue layers in the intestinal wall (the outer serous layer, the *tunica muscularis*, the *submucosa* and the *muscularis mucosae*) were intact. In some cases, micromorphological changes were developed similarly in the different groups.

In the Fabricius gland no pathological changes were found in general. The wall of the organ is covered by intact cylindrical epithelium inside and adventitia rich in adipocytes outside. In the propria layer of the mucosa, the follicles were filled with lymphocytes. The thickness of the muscle layer was adequate. Thus, healthy morphology was observed in each group.

The histological structure of *Thymus* was normal.

3.6. Microbiological tests

There was no *Salmonella spp*. isolated in the excreta samples during the test.

Clostridium perfringens was detectable in each group, but with different frequency and cell count. At the beginning of the experiment, the highest contamination was detected in the KO group, which decreased in time and was no longer detectable in any sample by day 42. In the PO and KOPO samples, the cell count was already lower even at the first sampling than for the KO and was no longer detectable after that. In the NO group, bacteria were detectable throughout the study and the degree of contamination increased in time.

The presence of *Coliform* microbes was the lowest at the time of the first sampling. It increased in time, except in the NO group, where the cell count was lower than the average value at the second and fourth samplings.

The results of the total germ count test were similar in each group and at each sampling. However, in the samples collected on day 42, the total germ count was the highest in the PO and NO groups and the difference was significant compared to KO samples. Moreover, the NO values were significantly beyond even the KOPO and control values as well.

4. CONCLUSIONS AND RECOMMENDATIONS

4.1. CONCLUSIONS

Feed tests

Due to the vegetable fat source supplementation used in the experiment the fat content of the experimental diets has increased more than twice, and consequently the apparent metabolizable energy content became almost 1MJ higher, while the crude protein content was reduced a bit. As a result, while the protein to energy ratio in the control feed was 15.1 g/MJ, it was between 13.2-13.9 g/MJ in the experimental groups (KO-13.5, PO-13.5, KOPO- 13.9, NO-13.2). This difference is significant from a practical point of view, as based on this, reduced feed intake might be predicted in the experimental groups, which may jeopardize the adequate protein supply. Otherwise, the nutrient composition of the experimental diets is considered to be the same.

The fatty acid composition of the coconut and palm oil used in my study was similar to the one reported in the literature (Bhatnagar *et al.*, 2009, Chowdhury *et al.*, 2007). Although, some differences might occur depending on the production area, or on the cultivar, these do not result in significant difference in the overall fatty acid composition (Marina *et al.*, 2009, Montoya *et al.*, 2014). In accordance with this, the dominant fatty acid of the coconut oil used in my tests is lauric acid (C12:0), while in palm oil the proportions of palmitic acid (C16:0) and oleic acid (C18:1n9t) are outstanding. The sunflower oil was used as a positive control, and it is rich in linoleic acid (C18:2n6c), but the ratio of oleic acid is also significant (C18:1n9t). Due to the different composition of the oil sources, the fatty acid composition of the experimental diets also varied. Htin *et al.* (2007) reported similar magnitude differences, when they studied the effects of 8% inclusion of coconut fat and palm oil among other fat supplements.

Production parameters

In my experiment, I primarily wanted to investigate the effect of fat sources on the physiological and meat quality parameters, however, performance data were also collected regularly. Although, due to the small number of individuals the experiment can only be considered as a model, and precise conclusions regarding production traits cannot be made, I still consider important to communicate these results and based on them to evaluate the effects of coconut oil and palm oil on the performance.

As a result of fat supplementation, the metabolizable energy content of the experimental diets increased and the protein/energy ratio decreased compared to the control, but at the same time the overall nutrient composition of the four fat supplemented diets was almost the same. So,

when comparing the results of the production parameters, focus was directed primarily on the treatment groups.

In my study, when coconut, palm oil and combined supplements were added, feed intake was similar to that of the control, while the birds consumed less feed as a result of sunflower oil supplementation. This experience is opposite to several literature data. Thus, for example, Dong and Thu (2021) reported gradual decrease in feed consumption with increasing coconut oil addition in the diet. Rahman *et al.* (2012) also experienced reduced feed intake when added 5% palm oil in the diet. At the same time, Khatun *et al.* (2017) reached contrary results, as they revealed lower consumption when sunflower oil was added in the diet compared to the same dose of palm oil supplementation. This finding agrees with my results. According to Wang *et al.* (2015), coconut oil supplementation does not affect the animal's feed intake, which is also consistent with my own data, although they only worked with a 1.5% inclusion rate. However, Elewa *et al.* (2023) reported an increase in feed intake with coconut oil supplementation. Most of the authors attribute the reduced consumption to the fact that the chicken adjusts its feed intake to the energy concentration of the feed (Fisher and Wilson, 1974), i. e. the higher the energy concentration of the diet the lower the consumption. In my study, this finding was only confirmed in the group of sunflower oil supplementation, since while the energy content of the feed was elevated with almost 1 MJ in each treatment group compared to the control, the feed intake was reduced only in that group. However, as the feed consumption was not measured individually, i. e. statistical analysis was not possible for this parameter, therefore a clear assessment of the differences between the groups is not possible.

No significant differences were experienced due to vegetable oil addition in the body weight and weight gain between the control and the individual experimental groups, or among the treatments. The related literature is contradictory, as Attia *et al.* (2020) reported some improvement in growth in the first period of fattening as a result of coconut oil supplementation. Similarly, in their experiment with coconut oil, Dong and Thu (2021) found positive effect on weight gain up to 6% inclusion rate, while above this, the positive effect disappeared, and the performance declined. According to other sources, palm oil supplementation does not affect body weight and weight gain of birds (Panigrahi and Powell, 1991; Onifade and Babatunde, 1998; Sundu *et al.*, 2005). Furthermore, the addition of 4% palm oil to the diet had adverse effect on the growth of the birds on the 2^{nd} and 4^{th} week of fattening (Rahman *et al.*, 2012). Controversially, Khatun *et al.* (2017) reported that sunflower oil supplementation resulted in increased body weight and weight gain, using

6% supplementation in their experiment. I did not experience any of these changes in my experiment, and in fact, based on my results, 5% coconut or palm oil supplementation have some benefit on live body weight and weight gain compared with sunflower oil.

In my study, coconut fat and sunflower oil supplementation resulted in low feed conversion ratio, but this cannot be considered beneficial, as parallelly the final weight was low. Rahman *et al.* (2012) did not experience similar effect when coconut oil was used for supplementation, while Dong and Thu (2021) reported gradual improvement in FCR in parallel and the effect was dose dependent. In my experiment, the addition of palm oil caused deterioration in feed efficiency, while in some literature sources it was reported that palm oil had no effect (Ayed *et al.*, 2016) or it even resulted in improvement (Nwoche *et al.*, 2003). Considering all the production results, the differences in FCR in my experiment are thought to occur primarily due to differences in feed intake. However, in the absence of statistical evaluation, clear conclusions cannot be drawn.

No significant effect of different oil supplements could be detected in the relative organ and breast yield in my study. In terms of its tendency, however, it is worth noting that the lowest relative breast weight was achieved with sunflower oil. Although several publications are available (Attia *et al.*, 2020, Wang *et al.*, 2015) in which the evolution of the relative mass of individual internal organs was examined, or breast yield related to oil supplementation was evaluated, the results are not consistent, or they cannot be compared with my experiment, as measurements were usually made only at the end of the fattening period. Nevertheless, in the mentioned researches, the different oil supplements did not cause any difference in organ weights, which is in line with my own tests, since the weight of the examined organs was similar in each group on day 42.

I consider it important to note that although the significant differences found in my experiment can be verified statistically, their value from a practical point of view is insignificant, as the difference between the treatments is only 0.2-1%. Due to the small number of individuals, the experiment can be considered to be a model, so the production parameters serve only as guidelines.

Meat quality parameters

The optimal pH value of chicken breast ranges from 5.7 to 6.1 (Qiao M. *et al.*, 2001, Van Laack *et al.*, 2000). After slaughter, however, it moves on a much wider scale and can even reach the value of 6.7 (Ristic and Damme, 2010). However, in high-quality meat, the pH drops rapidly after slaughter, so it reaches usually already the optimum range within 6-8 hours after slaughter, but, due to the changes caused by aging, for technological

reasons, pH measured after 24 hours cooling is considered as final one in practice (Petracci and Baeza, 2009). When the pH is out of the optimum range, thus the meat becomes more or less acidic than it is recommended quality problems might occur. Since the optimum pH of Pseudomonas, which is the most common reason of chicken meat spoilage, is between 6.0 and 8.0, to prevent bacterial growth it is extremely important to reduce pH below 6.1 as soon as possible after slaughter (Katyio *et al.*, 2020). According to observations, an insufficiently acidic pH primarily accelerates the rate of bacterial growth (Newton and Gill, 1981). At the same time, the excessively acidic pH (pH<5.7) also enhances bacterial spoilage, as the water-holding capacity of the meat decreases, and thus the cells proteins are released providing perfect media for certain bacteria to grow. In addition, when the pH is too acidic capability to process the meat might also be affected- Thus during marinating, it can absorb less marinade, or cooking loss might be increased (Northcutt, 1999; Allen *et al.*, 1998).

Based on the results of my experiment, the used fat sources did not have any negative effect on the pH of breast fillets, as though there were significant differences among the groups, but all the meat samples at each sampling had pH within the optimal range after 24 hours cooling. However, it is also worth to note that on day 35 and 42, the two samplings which have practical importance, the optimal pH was reached within 45 minutes after slaughter in each treatment group, which is a benefit over the control. These findings are consistent with the results of Khatun *et al.* (2017), who used 6% coconut or sunflower oil which resulted in similar values (5.78-5.80). Same results were reported by Ogunwole *et al.* (2016), who measured pH=5.91 with 2% coconut oil supplementation. At the same time, Prayitno *et al.* (2010) have found dose dependent effects on *post mortem* pH development when coconut oil (0-2%) was supplemented, and at 1% inclusion rate the pH fell out of optimal range, while at 2% it was as high as 6.25.

Examining the colour parameters of the meat no difference was found among the samples due to various fat and oil supplementation. This result slightly contradicts the literature data that pH influences the colour of meat, primarily its lightness (Allen *et al.*, 1998). This might be because though there were statistically significant differences in the pH values among the groups, each value was within the normal range, so this did not significantly affect the meat quality parameters of practical importance. The results published in the literature are contradictory considering meat colour and the individual data vary on extremely wide spectrum. In certain researches, like in my experiment, different fat sources did not influence the development of meat colour (Abdullah *et al.*, 2010; Prayitno *et al.*, 2010). In other studies, meat became significantly lighter and yellower as a result of palm kernel oil

supplementation compared with coconut oil addition (Ogunwole *et al.*, 2016). Altogether, the similar development of the meat colour in the treatment groups can be considered to be favourable, since no adverse effect in terms of consumer perception occurred.

In my experiment the water-holding capacity of the breast fillets was improved significantly with age, regardless of the treatment. This experience is in line with the findings of Baéza *et al.* (2012. This is a crucial trait in terms of shelf life and processing quality of the meat. On the one hand, it affects the juiciness and thus its organoleptic value. On the other hand, it determines the juice absorption capacity of the meat, so the efficiency of marination. The fact that the water-holding capacity of meat increases with age can be decisive in determining the optimal slaughter time.

When comparing the treatments, similar values were measured in each group at a given time in general, but on days 28 and 35 drip loss in the treatment samples was higher than in the control. However, on day 42, practically the same drip loss was observed in each group. Comparing these results with literature data, it can be established that drip loss is highly variable (Soeparno, 1992a; Prayitno *et al.*, 2010). My results are similar to those described by Ogunwole *et al.* (2016). They measured 10.21% loss, when 2% coconut oil was added to the diet. Khatun *et al.* (2017) experienced the same tendency, as well.

It is well known that meat pH and water holding capacity are related, i.e. lower pH is associated with lower water holding capacity (Barbut, 1993). This relationship was evident until the slaughter on day 35.

Kitchen processing losses, were characterized as the weight loss measured during thawing the frozen breast fillet, during frying the samples and cooling them to room temperature. In the literature, kitchen losses often referred to as cooking losses. As some other meat quality parameters, kitchen loss may vary within extremely wide range. It is affected by age, as e.g. Soeparno (1992a) reported that the cooking loss of broiler meat was 24.89% and 34.57% at 6 and 7 weeks of age. While at a certain age it was found to vary between 15% and 40% depending on the feed (Soeparno, 2005). When I compared the thawing, frying and cooling loss results with literature data of cooking loss I came to the conclusion that the combined value of losses I measured is the best comparable with it. Taking this into account, on day 28 losses developed similarly in each group, while on day 35 hectic pattern was found with large individual standard deviation. At this time, positive effect of the coconut oil was observed. On day 42, the differences among the groups were less pronounced, but the highest loss was measured when coconut oil was added, and each treatment group has shown elevated loss (KO-19.32%, PO-18.74%, KOPO-15.47 %, NO-

19.39%) compared to the control (12.58%). These results are not totally consistent with literature data. According to Prayitno *et al.* (2010), for example, relations were opposite when 2% coconut oil supplementation was used, i.e. losses were lower in the treatment group compared to the control. Ogunwole and his colleagues compared the effects of coconut oil and palm kernel oil supplementation and found that palm kernel oil resulted in a significantly greater loss than in the case of coconut oil supplementation, however, the dose in my experiment was 2.5 times higher. The reason behind the increased kitchen losses – similar to drip loss – is thought to be the low initial pH of the meat. This agrees with Jankowski *et al.* (2012) statement that the change in drip and kitchen processing losses shows similar pattern. Based on literature data, pH and drip loss, as well as pH and cooking loss, show moderate, negative correlation. This is because as the pH decreases, proteins of the myofibrils reach their isoelectric point, and consequently the activity of the ion pump decreases, which results in simultaneous decline in the amount of water holding capacity of the cells (El Rammouz *et al.*, 2004). Accordingly, the more acidic pH measured in the PO, KOPO and NO groups compared to the control on day 35 resulted in increased dripping and kitchen losses. However, on day 42, this relation was less clear.

The normal shear force value of broiler chicken breast fillet is between 1.5-2.5 kg/cm^2 based on previous own tests and literature data. Lyon *et al.* (2004) reported shear force between 1.82 kg/cm^2 and 2.19 kg/cm^2 in chicken meat. In the present experiment similar values were measured in each group. During the test period, the shear force value was gradually increased with age in the control samples, which is in agreement with the results of published studies (Park *et al.*, 2021). However, in the groups with oil supplementation significant increase was observed on day 35, followed by declining on day 42. On the former date, the control breast fillet was softer than the meat of each treatment groups, while at the end of the experiment, however, the meat texture in the treatment groups was similar to the control, and the fillets were softer due to palm oil addition than in groups of coconut or combined oil supplementation. The result agrees with literature data reported softening effect of palm oil on breast fillet (Ogunwole *et al.*, 2016; Khatun *et al.*, 2017). However, Prayitno *et al.* (2010) found significant improvement in meat texture due to coconut oil supplementation, which is contradictory to my results.

Considering chemical composition of the meat, though statistically significant differences were found among some groups these do not have any practical relevance as the differences were within one percent. Basically, fat supplementation did not cause any change in the lipid content of the meat, but the fatty acid composition was modified by the fat source.

This result is consistent with the results of similar studies published (Kralik *et al.*, 2007; Valavan *et al.*, 2006; Crespo and Estevé Garcia, 2001). In my experiment, the most prominent effect was found in the coconut oil group, where the lauric acid content was increased markedly. Due to sunflower oil supplementation the proportion of oleic acid and linoleic acid increased, but the change was less pronounced, while no significant change in fatty acid composition was found due to palm oil addition. The reason of this latter finding might be the fact that the breast meat originally has relatively high palmitic acid content, so the further "enrichment" is less effective than, to improve the original trace amount of lauric acid with coconut oil supplementation.

The results of the correlation analysis related with the meat quality parameters are generally consistent with those described in the literature. However, according to Le Bihan-Duval *et al.* (1999), the pH value measured 24 hours after extermination has negatively correlated with meat lightness (L*) (-0.60) and dripping loss (-0.40), while in my experiment the lightness (L* value) of the meat did not show any correlation with the mentioned parameters, regardless of the treatment, but drip loss has shown positive correlation with the pH measured 45 min after slaughter (0.5432), with thawing (0.3263) and cooling loss (0.3731).

In addition, according to Hoffman *et al.* (2003) there is negative correlation between the pH value and softness of meat. Similar negative correlation was revealed in my experiment regardless the treatment between the texture and the pH (-0.2332) measured 45 min after slaughter and the pH (-0.2811) measured after 24 hours cooling.

Altogether, it can be concluded that the fat sources I used did not have any significant negative nor positive effects on the sensory and physical quality parameters of the meat. At the same time, it is important to note from nutritional and physiological point of view that the ratio of saturated fatty acids was gradually increased parallel to the duration of coconut oil supplementation, while this was not experienced with palm oil.

Biochemical parameters

Primarily, the blood parameters used to characterize fat metabolism (cholesterol and triglycerol concentration) did not show drastic changes due to fat supplementation. The triglycerol concentration developed in the same manner in the different groups during the entire experimental period. At the same time, plasma cholesterol concentration has shown some response to fat supplementation. Thus, cholesterol concentration was higher in the treatment groups than in the control even after one week of supplementation, and the difference increased as time was passing by. However, the difference was proven to be significant only between the coconut oil and the control groups. Considering treatment groups sunflower

oil was the least efficient to modify these parameters. Similar results were reported by Khatun *et al.* (2017), when 6% sunflower or palm oil was added in the diet and also by Velasco *et al.* (2010) and Viveros *et al.* (2009). Donaldson *et al.* (2014), have found elevated plasma cholesterol concentration due to high-dose fat supplementation in different poultry species, too.

However, the magnitude of the changes in the studied lipid parameters indicates that the applied fat additives did not affect significantly fat metabolism of the birds.

Changes found in the blood plasma antioxidant parameters on week three, refers to slight induction of the glutathione redox system regardless of the treatments. Blood plasma is the first line of defence, and all the environmental effects cause fast changes in it. As in my experiment similar changes were found in each group, it is believed that some kind of oxidative effect could occur in the birds. This is indicated by the increased malonyl dialdehyde concentration measured at the second slaughter and, in parallel, by the elevated glutathione peroxidase activity, which continued to rise till the third week of treatment. However, based on the available data, the reason cannot be identified properly. Considering the differences among the treatments, the most pronounced deviations were identified on day 42. The studied antioxidant status parameters in the treatment groups were higher than in the control except that of the PO. However, the difference can be considered significant only between the PO and KOPO groups. The reduced glutathione concentration values were changing linearly with the glutathione peroxidase activity in each group. This is a clear evidence of the close relations of the two parameters, since GSH acts as the co-substrate of the enzyme (GSHPx). The reactions in the PO and KOPO groups drives to the conclusion that palm oil supplementation might enhance the reaction of the antioxidant defence system.

The parameters used to characterize the redox status in the red blood cell (RBC) hemolysate were much more uniform than that of the blood plasma. Since red blood cells are more stable than blood plasma, more drastic intervention is required to identify changes eve there. Nevertheless, pronounced differences occurred on day 35 even in RBC. In general, increased GSHPx activity was revealed in each treatment group, while the reduced glutathione concentration dropped when coconut oil was included in the diet. However, this effect disappeared by day 42. It is also important to note that in this case the malondialdehyde concentration remained stagnant throughout. Thus, the observed differences in activity cannot be traced back to external oxidation effects.

There was no significant difference among the groups when oxidation (amount of conjugated diene and triene, as well as malonyl dialdehyde

concentration) and antioxidant capacity parameters were measured in the liver. Thus, the fat sources did not cause significant oxidation risk compared to the control. This is in line with the findings that no difference was identified in the antioxidant parameters among the groups. It is also important to note that reduced glutathione concentration and glutathione peroxidase activity decreased over time when coconut oil was added to the diet, while similar, but less obvious tendencies were found in the combined treatment. These values were stagnant when palm kernel oil was included in the diet, which is in line with the studies of Long *et al.* (2019), who found no significant induction of the glutathione redox system with 2, 4, 6% palm oil supplementation.

In summary, studying the antioxidant defence parameters the applied fat sources did not pose any significant oxidation risk, or the glutathione redox system of the birds was able to neutralize the oxidative effects. The fact that the glutathione peroxidase activity and the reduced glutathione concentration showed changes mostly and solely in the blood plasma, while stable and almost identical values were measured in the liver in each group, indicates that the additional oil supplementation in optimal conditions might cause slight oxidative induction, but it can be managed by the first line of the defence(antioxidant enzymes of the blood), while the liver is not affected. Thus, oil supplementation alone does not serve as a predisposing factor of oxidative stress.

At the same time, different sources of fat influence the status of the glutathione antioxidant system. This can be confirmed by the fact that due to coconut oil supplementation both the reduced glutathione concentration and the glutathione peroxidase activity are declining over time. This might be related to the antioxidant character of coconut oil (Nevin and Rajmohan 2006; Ghani *et al.*, 2018), which is presumably the result of its polyphenolic components. Similar results were found in atherosclerosis studies in human experiments (Sabitha and Vasudevan, 2010), however, I did not find such studies in the literature with poultry.

Histological examinations

Under certain conditions, excessive fat intake results in the accumulation of fat in the liver resulting first in lipid infiltration, then haemorrhages, lipotoxicity and inflammatory processes might occur (Guo *et al.*, 2021). In my experiment, sporadic signs of fat accumulation and lymphocyte invasion (which may indicate inflammation) were identified in each group, including the control. Due to the low number of samples clear conclusion cannot be drawn, however, as the histological structure was similar in the control and the treatment groups the applied fat additives seems to be harmless in liver when the doses used in the experiment are

applied. This is also confirmed by the fact that no marked changes were recorded in the antioxidant parameters.

Considering jejunum samples, only some dilatated Lieberkühn crypts were found sporadically, containing lymphocyte debris, which might indicate initiation of inflammation. Similar processes were observed in the *bursa Fabricii*, while no abnormalities were revealed in the *thymus*.

Based on the results of histological studies, the tested fat sources did not cause pathological changes in the tested organs and tissues at the applied dose.

Microbiological parameters

Several studies have been published so far reported evidence on the inhibitory effect of medium-chain fatty acids (lauric acid, caprylic acid) on bacterial growth (Widianingrum *et al*., 2019; Lalouckova, 2019). In these, mainly *in vitro* experiments the bacteriostatic effect of these fatty acids was confirmed on gram-positive bacteria. In my study some microbiological parameters were analysed in the excreta. Based on the results no pronounced antimicrobial effect was proven against Gram-negative *coliform* bacteria. At the same time, both the occurrence and the colony count of Gram-positive *Clostridium perfringens* decreased in time course, when single coconut or palm oil was included in the diet, as well as when combined treatment was used. This inhibitory effect is supported by the histological analysis of the jejunum samples, in which only a few enlarged crypts filled with cellular debris referring to the initial symptoms of intestinal inflammation possibly induced by these pathogens were found. The background of this effect may lie in the bacteriostatic effect of medium carbon chain fatty acids (lauric acid, caprylic acid).

4.2. Recommendations

Based on my results, the following recommendations are outlined:

- It is advisable to validate the results of the model experiment in a a medium or large-scale studies. Large number of individuals and samples can provide sufficient reliability and validity to confirm preliminary data of the collected production parameters.
- To investigate the effect of fat and oil supplementation over time, it is recommended to set up a similar model experiment, in which duration of the oil supplementation varies (it would be 1, 2 and 3 weeks long periods), so that all birds would be exterminated on the 42nd day of life. In this way, meat quality parameters would be better comparable according to consumer considerations.
- At slaughters, it would also be advisable to examine how the composition of the carcass and the amount of abdominal fat develop.

- When measuring kitchen losses, it is recommended to analyse the fat content, also. During frying, not only the moisture content of the meat decreases, but also a part of the fat may melt, which, according to my assumption, can also be related to the changed fatty acid composition caused by the additional oils.
- It is recommendable to create further tests to study the relation between meat texture and fat supplementation The purpose of a new experiment would be to reveal the metabolic mechanism behind the experience that the addition of 5% coconut or palm oil resulted in improved meat texture, while in combination the beneficial effect disappeared.

5. NEW SCIENTIFIC RESULTS

1. Studying the effects of 5% coconut or palm oil supplementation, it can be stated that the additive fat sources do not significantly affect the fat content of the meat, but at the same time they cause change in the fatty acid composition of chicken meat in accordance with the fatty acid composition of the fat source. In order to achieve this effect, it is necessary to feed the fat sources for two weeks at least when using the same inclusion rate I used.
2. Considering texture of the chicken meat, improved quality is realised after 3 weeks of 5% single coconut or palm oil supplementation by the age of 42 days. However, this effect disappears when the two oils are used in combination.
3. Five percent additive fat source results in increased frying and cooling loss, when the meat of 42 days old chicken is processed, and the extent of the losses is related to the type of the fat source..
4. It was revealed that supplementing broiler chicken feed with 5% single coconut or palm oil or with a 2.5-2.5% mixture of the two oil sources significantly affect the glutathione antioxidant defence system, it results in slight induction of the glutathione peroxidase in the blood plasma, which enables to keep lipid peroxidation processes at physiological level.
5. Based on my study that the activity of the glutathione-redox system declines in time course, when coconut fat is included in the diet, which might cause reduced responsiveness of the system in case of potential oxidative stress.
6. It is revealed that the presence of gram-positive *Clostridium perfringens* can be reduced by adding coconut oil to the diet of chicken in 5% inclusion rate.

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Scientific communication in journal:

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