Abstract: Influence of Ag nanoparticles, ATP and biocomplex of Ag nanoparticles with ATP on morphology of chicken embryo pectoral muscles.

INTRODUCTION
Increase of genetic potential of fast growing chicken broilers to enlarge size of muscles may lead to decrease quality of the meat because of non-adequate amount of nutrient and energy, stored within the eggs, for providing optimal development. Deliver to the chicken embryo additional amount of energy as ATP or ATP attached to the nanoparticles of silver, as a transporting molecule, may promote growth and development of breast muscle. The objective of the investigation was to evaluate the effect of Ag nanoparticles and ATP, administrated in ovo to the embryo, on the morphology of pectoral muscle. The fertilised eggs of Ross 308 (160) were divided into four groups (4 × 40 eggs): without injection (control), with injection of hydrocolloid of Ag nanoparticles (Nano-Ag), with injection of hydrocolloid of adenosine triphosphate (ATP) and with injection of Nano-Ag conjugated with adenosine triphosphate (Nano-Ag/ATP). At day one of incubation, the eggs were injected into the air sac with 0.3 ml of experimental solutions. Chicken embryo morphology was evaluated according to the Hamburger-Hamilton standard stages of embryo development, furthermore, pectoral muscle was visualized by TEM. Results showed that Nano-Ag, ATP and Nano-Ag/ATP did not affect negatively growth and development. However, ultra morphology of the cross section of embryo pectoral muscles was better structured and muscle was more dense with myofibers when ATP and Ag nanoparticles were applied. The results indicate that application of Nano-Ag and ATP in ovo can affect morphology of breast muscle, but not affecting embryo growth.

Key words: silver nanoparticles, pectoral muscle, ATP, embryo, chicken, transmission electron microscope.
method with injection to albumen or air sac, may be broken down with enzymes presented within eggs or not properly distributed. It can be supposed that applying nanoparticles as an ATP molecule delivery platform may support ATP distribution within eggs. Nanoparticles, with their smaller size, allow for penetration into the tissue and going even deeper, crossing cell membranes (Debbage and Thurner 2010, Hotowy et al. 2012, Peng et al. 2012). Some studies demonstrated that the Ag nanoparticles were non-toxic, antibacterial (Sironmani and Daniel 2011), furthermore, upregulated expression of anabolic genes – FGF2 and VEGF in chicken embryo muscles (Hotowy et al. 2012).

We hypothesized that ATP attached to Ag nanoparticles would be delivered to the muscle cells, as a promoting growth and development of embryo breast muscle. The objective of the investigation was to evaluate the effect of Ag nanoparticles, ATP and ATP conjugated with Ag nanoparticles, administrated to the chicken in ovo, on the morphology of pectoral muscle.

MATERIALS AND METHODS

The fertilised eggs of Ross 308 chicken were obtained from a commercial hatchery. 160 eggs were randomly divided into four groups (4 × 40 eggs): without injection (control), with injection of hydrocolloid of Ag nanoparticles (Nano-Ag), with injection of hydrocolloid of adenosine triphosphate (ATP) and with injection of hydrocolloid of Ag nanoparticles conjugated with adenosine triphosphate (Nano-Ag/ATP). At day 1 of incubation, the eggs were numbered, weighed (60 ±1.36 g) and injected into the air sac with 0.3 ml of experimental solutions using a sterile 27 gauge, 20 mm needle. Immediately after the injection, the hole was sealed with hypoallergenic tape and the eggs were placed into an incubator. The eggs were incubated for 20 days under standard conditions (temperature 37.8°C, humidity 55%, turned once per hour during the first 18 days, at a temperature of 37°C and humidity 60% from day 19).

The embryos were decapitated and evaluated on day 20 of incubation. The morphological structure was compared with the standard described by Hamburger and Hamilton (1951), samples of the pectoral muscles were fixed in glutaraldehyde for electron microscopy preparation.

The hydrocolloid of Nano-Ag, at the concentration of 50 ppm and size of 2 to 35 nm was obtained from Nano-Tech (Warsaw, Poland). Pure ATP (Merck, Germany) was dissolved in distilled water at the concentration of 0.04 mg per 1 ml. ATP was conjugated with AgNano using sonication for 30 min at 15°C in an ultrasonic bath.

For observations with transmission electron microscope (TEM), tissues were cut into pieces of about 1 mm³ and fixed (60 min) in 3% glutaraldehyde solution (Merck) in 0.1 M sodium phosphate buffer (pH 7.2). Samples were then rinsed (2 × 5 min) in the same buffer and transferred to a 1% osmium tetroxide solution in 0.1 M phosphate buffer (pH 6.9) for 1 hour. Subsequently, the samples were rinsed in 0.1 M sodium phosphate buffer (5 min), dehydrated in an ethanol gradient (50–99%), and impregnated with Epon embedding resin (Merck). The blocks were cut into ultrathin sections.
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Chicken embryo breast muscle tissues were visualized by TEM, which allows observation of the ultrastructure of pectoral muscle (Figure 1). Pictures of the muscle cross section showed some differences between the groups. The areas of muscle fibers of the chicks from the control group were the smallest and fibers were placed more loosely, space between myofibers was bigger and also the number of myofibrils seen within cytoplasm was smaller. Furthermore, myofibrils were located freely and spaces between them were bigger compare to the other groups. Pictures of muscles from the control embryos may point to a non-adequate organization and an immature structure of the tissue. The muscle from treated groups were better developed, myofibers were bigger and placed more compressed. However, in embryos administrated with ATP myofibrilles were more dense and their structure was more concentrated. The quantitative measurements demonstrated that embryos from the control group had significantly the highest number of muscle cells per square unit compared to the rest of groups (Table 1). The biggest area of pectoral muscles fibers was observed in the treatment groups, especially with Nano-Ag/ATP and ATP. For the other side, the number of nuclei per quadratic surface (1200 μm²) was at the same level in all groups. Furthermore, the number of nuclei per cell was bigger in the treatment groups, the biggest in ATP administrated embryos. In the light of obtained results it can be supposed that the better morphology of the chicken embryo muscles treated with ATP and Nano-Ag/ATP was caused because of ATP stimulating DNA synthesis, protein synthesis and also acting syner-
FIGURE 1. TEM image of pectoral muscle (cross-section) in the Control group and groups with treated with Ag nanoparticles (Nano-Ag), adenosine triphosphate (ATP) and in combination of Nano-Ag with ATP (Nano-Ag/ATP). (A) Single muscle fibre, (B) Endomysium, (C) Myofibrils, (D) Nucleus

TABLE 1. Morphometry and average weight of embryos and pectoral muscles of chicken

<table>
<thead>
<tr>
<th>Specification</th>
<th>Treatment</th>
<th>Control</th>
<th>Nano-Ag</th>
<th>ATP</th>
<th>ATP/Nano-Ag</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>mean</td>
<td>SEM</td>
<td>mean</td>
<td>SEM</td>
<td>mean</td>
</tr>
<tr>
<td>Cell number</td>
<td>17^A</td>
<td>0.26</td>
<td>10^B</td>
<td>0.25</td>
<td>9^B</td>
</tr>
<tr>
<td>Number of nuclei</td>
<td>7.3</td>
<td>0.67</td>
<td>8.2</td>
<td>0.53</td>
<td>8.9</td>
</tr>
<tr>
<td>Fiber area</td>
<td>38^A</td>
<td>1.62</td>
<td>79^C</td>
<td>2.57</td>
<td>50^B</td>
</tr>
<tr>
<td>Embryo, % e.w.</td>
<td>78^A</td>
<td>0.6</td>
<td>77^A</td>
<td>0.5</td>
<td>67^B</td>
</tr>
<tr>
<td>Muscle, % b.w.</td>
<td>0.81</td>
<td>0.026</td>
<td>0.80</td>
<td>0.041</td>
<td>0.76</td>
</tr>
<tr>
<td>Number of nuclei per 1 cell</td>
<td>0.43</td>
<td>0.80</td>
<td>0.95</td>
<td>0.64</td>
<td></td>
</tr>
</tbody>
</table>

^1Percentage of egg weight; ^2percentage of body weight.
A, B, C – Within rows: means with different superscript differ significantly (P < 0.05).
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consequence, the adenosine triphosphate had influence on the morphology of pectoral muscles. Nanoparticles of silver did not improve activities of ATP, however, as a gene expression activator may support the process.

CONCLUSIONS

ATP, silver nanoparticles and ATP conjugated with Ag nanoparticles, given with in ovo methods to the chicken embryo did not negatively influence growth and development. The cross section of chicken embryo pectoral muscles was better structured, matured and more dense with myofibers when ATP and Ag nanoparticles were applied. The results indicate that application of Nano-Ag and ATP in ovo can affect morphology of breast muscle furthermore, but having no influence on embryo growth.

REFERENCES


Streszczenie: Wpływ nanocząstek Ag oraz ATP i biokompleksów nanocząstek Ag z ATP na morfologię mięśnia pierśowego zarodka kury. Zwiększenie potencjału genetycznego szybkożrących ras kur do zmniejszenia wielkości mięśni, może prowadzić do zwiększenia jakości mięsa, jako następstwa względnego niedoaboru składników pokarmowych i energii zgromadzonych w jaju i warunkujących optymalny rozwój. Dostarczenie zarodkowi kur nanocząstek srebra, jako molekule transportującej, może zapewnić właściwy rozwój mięśni pierśowych. Celem badań była ocena wpływu nanocząstek Ag i ATP, podawanych in ovo, na morfologię mięśni pierśowych zarodków kury. Zapłodnione jaja kur Ross 308 (180 sztuk) podzielono na 4 grupy (4 × 40 sztuk): kontrolną, poddaną iniekcji Nano-Ag z przyłączonymi cząsteczkami ATP (Nano-Ag/ATP). W pierwszym dniu inkubacji do komory powietrznej jaj wstrzykiwano 0,3 ml eksperymentalnych roztworów. Morfologia zarodków kury była oceniana według standardu Hamburgera i Hamiltona, ponadto próbki mięśnia pierśowego były oceniane za pomocą transmisyjnego mikroskopu elektronowego (TEM). Wyniki wykazały, że Nano-Ag, ATP i Nano-Ag/ATP nie wpłynęły negatywnie na wzrost i rozwój zarodków. Ocena ultra morfologii przekroju poprzecznego wycinków mięśni pierśowych wykazała lepsze wykształcenie mięśni pierśowych, większą gęstość włókien mięśniowych u zarodków, którym podawano Ag i ATP. Wyniki wykazały, że podawanie Nano-Ag i ATP in ovo może wpływać korzystnie na morfologię mięśni, nie działając negatywnie na wzrost zarodków.

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