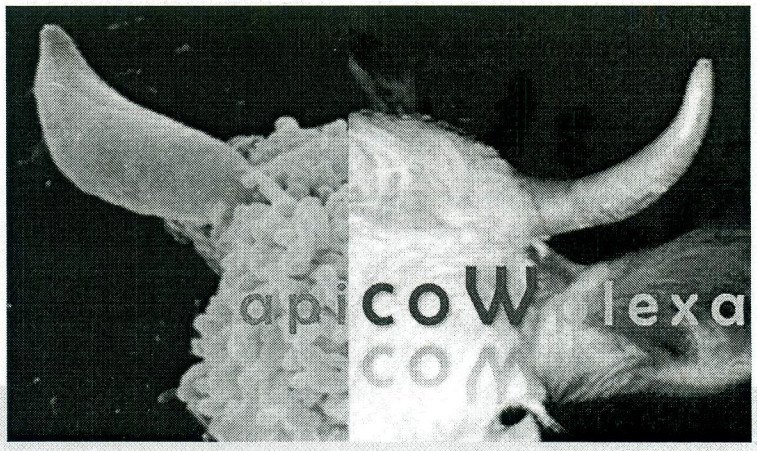
proceedings



2nd International Meeting on
Apicomplexan Parasites in Farm Animals

October 31 – November 2, 2013

Kuşadası, Türkiye

Effect of artemisinin on immune system in chickens: preliminary results

Adriana Györke[1]*, Loredana Pop[1], Anamaria Paştiu[1], Zsuzsa Kalmar[1], Mirabela Dumitrache[1], Flaviu Tăbăran[2], Mircea Mircean[3], Cristian Magdaş[1], Viorica Mircean[1], Vasile Cozma[1]

1 Parasitology and Parasitic Diseases Dept., University of Agricultural Science and Veterinary Medicine Cluj Napoca, Fac. Vet. Med., 3-5 Calea Mănăştur, 400372 Cluj-Napoca, Romania
2 Pathologic Anatomy and Necropsy Dept., University of Agricultural Science and Veterinary Medicine Cluj Napoca, Fac. Veterinary Med., 3-5 Calea Mănăştur, 400372 Cluj-Napoca, Romania
3 Internal Medicine Department, University of Agricultural Science and Veterinary Medicine Cluj Napoca, Faculty of Veterinary Medicine, 3-5 Calea Mănăştur, 400372 Cluj-Napoca, Romania
* titilincua@yahoo.com

We investigated the effect of artemisinin on immune system in broiler chickens following chronic oral intake by lymphocyte proliferation assay (mitogens: concavalin A, lipopolysaccharide, artemisinin), phagocytosis assay, evaluation of serum gamma-globulin (SGG) levels, and development of lymphoid organs (bursa of Fabricius, spleen, and thymus). The chickens received artemisinin (5, 50, and 500 ppm) in their diet from 18 to 46 day-old. The level of serum gamma-globulin was significantly higher in chickens in-feed treated with artemisinin than in untreated chickens. However, only in chickens treated with 5 and 50 ppm the level of SGG increased over time, while in chickens treated with 500 ppm decreased as in control group. The *in vitro* phagocytic activity was supressed by artemisinin only at 28 days after in-feed treatment with artemisinin, but without statistical significance comparing with control group. Interestingly, in variants without stimulation, and stimulation with lipopolysaccharide, in-feed treatment with 50, and 500 ppm artemisinin stimulated the *in vitro* phagocytic activity. As regarding the thymus weight we did not registered statistical significant difference among the groups, even chickens in-feed treated with 50, and 500 ppm artemisinin had a lower weight. Generally, the stimulation index of lymphocytes was higer in chickens treated with 50, and 500 ppm artemisinin and *in vitro* stimulated with concavalin A and lipopolysaccharide, but lower in case of *in vitro* stimulation with artemisinin. Over time, stimulation index decreased in all treated chickens, but greater in chickens treated with 50 ppm artemisinin, and *in vitro* stimulated with concavalin A and artemisinin, while in case of lipopolysaccharide increased. Also, the weight of bursa was significant lower in chickens treated with 50 ppm artemisinin, followed by chickens treated with 500 ppm artemisinin, comparing with untreated chickens. The spleen had a lower weight in chickens treated with 50, and 500 ppm artemisinin, but without any statistical significant difference. Artemisinin is relatively immunotolerant in broiler chickens, and further investigations are needed to make a final conclusion.

This work was supported by UEFISCDI, project number PN II-PCCA Type 2 110/2012.

EFFECT OF ARTEMISININ ON IMMUNE SYSTEM IN CHICKENS: PRELIMINARY RESULTS

Adriana Györke[1]*, Loredana Pop[1], Anamaria Paştiu[1], Zsuzsa Kalmar[1], Mirabela Dumitrache[1], Flaviu Tăbăran[2], Mircea Mircean[3], Cristian Magdaş[1], Viorica Mircean[1], Vasile Cozma[1]

University of Agricultural Science and Veterinary Medicine Cluj-Napoca, Faculty of Veterinary Medicine, 3-5 Calea Mănăştur, 400372 Cluj-Napoca, Romania
[1]Parasitology and Parasitic Diseases Department ; [2]Pathologic Anatomy and Necropsy; [3]Internal Medicine Department
***titilincua@yahoo.com**

INTRODUCTION

Artemisinin is a sesquiterpene lactone isolated from the Chinese herb *Artemisia annua*, and has been used for centuries to treat malaria. This compound has antiviral (Romero et al., 2006; Efferth et al., 2008), anti-cancer effects (Efferth, 2006) and antibacterial properties (Dhingra et al., 2000). Artemisinin was found to be effective in controlling poultry coccidiosis (Arab et al., 2006; Naidoo et al., 2008; del Cacho et al., 2010), and it is also efficient on *Schistosoma, Babesia caballi*, hepatic trematodes, trypanosomiasis and leishmaniosis (Utzinger, 2007; Kumar et. al., 2003; Keiser et. al., 2006; Mishina et al., 2007; Sen et al., 2010).

Artemisinin contains an endoperoxide bridge which by reductive breaking, operated by iron complexes, generates reactive radicals that would produce damage to the parasites (Galasso et al., 2007). It was also shown that treatment with artemisinin alters the process of oocysts wall formation in case of *Eimeria* (del Cacho et al., 2010). Although artemisinin has been proven to be safe in therapeutic doses, some researchers have shown side effects of this compound. Embryolethality, morphological abnormalities during early pregnancy, hormonal imbalances were induced by treatment with artemisinin in mammals (Boareto et al., 2008). In broiler chickens, artemisinin causes decreases in red blood cells count and hematocrit, anemia, kidney, liver and brain lesions (Arab et. al., 2009; Shahbazfar et. al, 2011). Effect on immune system was studied in a mouse model. The study showed that topical administration of artemisinin could supress CHS response. Artemisinin had an inhibitory effect on IL-17 production, diminished the level of IL-6 and enhanced TGF-β (Li et al., 2012).

The present study aimed to determine the effects of different doses of artemisinin on the immune system of broiler chickens following chronic oral intake.

REFERENCES

1. **Arab, H.A., Rahbari, S., Rassouli, A., Moslemi, M.H., Khosravirad, F.** Determination of artemisinin in *Artemisia sieberi* and anticoccidial effects of the plant extract in broiler chickens. *Trop Anim Health Prod*. 2006, 38, 497-503.
2. **Arab HA, Mardjanmehr SH, Shahbazfar A, Rassouli A, Abdollahi M, Nekouie O.** Toxicopathologic Effects of Artemisinin in Broiler Chickens Following a Single Oral Dose: An LD 50 Study. *Int J Poultry Sci*. 2009;8:808–812.
3. **Boareto A.C., Muller J.C., Bufalo A.C., Botelho G.G.K., de Araujo S. L., Foglio M.A., de Morais R. N., Dalsenter P.R.** Toxicity of artemisinin [Artemisia annua L.] in two different periods of pregnancy in Wistar rats, *Reprod. Toxicol.* 2008; 25,239–246
4. **del Cacho E., Gallego M., Francesch M., Quílez J., Sánchez-Acedo C.** (2010). Effect of artemisinin on oocyst wall formation and sporulation during *Eimeria tenella* infection. *Parasitol Int*. 2010, 59, 506-511.
5. **Dhingra, V., Pakki, S.R. & Narasu, M.L.** Antimicrobial activity of artemisinin and its precursors. *Curr Sci*. 2000, 78, 709-713;
6. **Efferth T.** Molecular pharmacology and pharmacogenomics of artemisinin and its derivatives in cancer cells. *Curr Drug Targets* 2006;7:407-21.
7. **Efferth, T., Romero M. R., Wolf , D. G., Stamminger, T., Marin, J. J. and Marschall, M.** The antiviral activities of artemisinin and artesunate. *Clin. Infect. Dis.* 2008, 47:804–811.
8. **Galasso V., Kovac B., Modelli A.** A theoretical and experimental study on the molecular and electronic structures of artemisinin and related drug molecules. *Chem phys*. 2007, 335,141–154.
9. **Keiser, J.; Utzinger, J.** Food-borne trematodiasis: current chemotherapy and advances with artemisinins and synthetic trioxolanes. *Trends Parasitol*. 2007, v.23, n.11, p.555-562;
10. **Kumar, S.; Gupta, A.K.; Pal, Y.; Dwivedi, S.K.** In vivo therapeutic efficacy trial with artemisinin derivative, Buparvaquone and Imidocarb Dipropionate against *Babesia equi* infection in donkeys. *J Vet Med Sci*. 2003, v.65, n. 11, p.1171-1177;
11. **Li T., Chen H., Wei N., Mei X., Zhang S., Liu DL., Gao Y., Bai SF., Liu XG., Zhou YX.** Anti-inflammatory and immunomodulatory mechanisms of artemisinin on contact hypersensitivity. Int Immunopharmacol. 2012; 12(1):144-50
12. **Mishina, Y.V., Krishna, S., Haynes, R.K., Meade, J.C.** Artemisinins Inhibit Trypanosoma cruzi and Trypanosoma brucei rhodesiense In Vitro Growth. Antimicrob. Agents Chemother. 2007, 51:1852–1854.
13. **Naidoo, V., McGaw, L.J., Bisschop, S.P., Duncan, N., Eloff, J.N.** The value of plant extracts with antioxidant activity in attenuating coccidiosis in broiler chickens. Vet Parasitol. 2008, 153, 214-219.
14. **Romero MR, Serrano MA, Vallejo M, Efferth, T, Alvarez M, Marin JJG.** Antiviral effect of artemisinin from *Artemisia annua* against a model member of the *Flaviviridae* family, the bovine viral diarrhoea virus (BVDV). *Planta Med* 2006;72:1169-74.
15. **Sen R., Ganguly S., Saha P.,Chatterjee M.** Efficacy of artemisinin in experimental visceral leishmaniasis. *Int J Antimicrob Ag*. 2010, 36, 43–49
16. **Shahbazfar, A.A., Mardjanmehr, S.H., Arab, H.A., Rassouli, A., Abdollahi, M.** Effects of artemisinin in broiler chickens following chronic oral intake. *Trop. Anim. Health Prod*. 2011, 43, 843-849.
17. **Utzinger J, Xiao SH, Tanner M, Keiser J.** Artemisinins for schistosomiasis and beyond. *Curr Opin Investig Drugs*, 2007;8:105-16.

MATERIAL AND METHODS

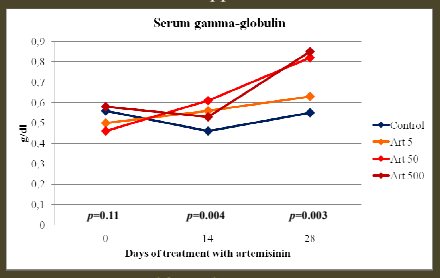
We used four experimental groups of 10 chickens (ROSS 308), divided in two replicates of five chickens (one females, and one males). One group received no treatment (control group) and three groups received artemisinin in their diet at 5, 50 and 500 ppm concentrations. The treatment started when chickens were 18 days and lasted for 28 days (46 days old). Blood and serum samples were harvested from each chicken before treatment and two times after treatment at 14 and 28 days. Finally, chickens were euthanized by cervical dislocation and lymphoid organs (bursa of Fabricius, spleen, and thymus) collected. Feed and water were provided *ad libitum*, and the lighting was continuous. The chickens were reared in cages. Artemisinin of 98% purity was purchased from INTATRADE Chemicals GmbH, Germany.

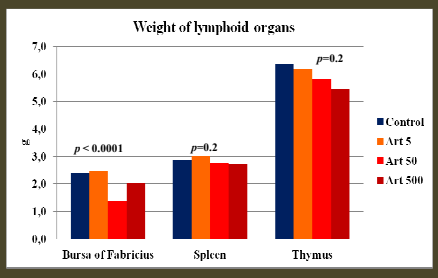
The effect of artemisinin on immune system in broiler chickens was assessed by lymphocyte proliferation assay using whole blood (mitogens: concavalin A, lipopolysaccharide, artemisinin), phagocytosis assay, evaluation of serum gamma-globulin levels, and development of lymphoid organs (weight).

RESULTS

Serum gamma-globulin levels and weight of lymphoid organs

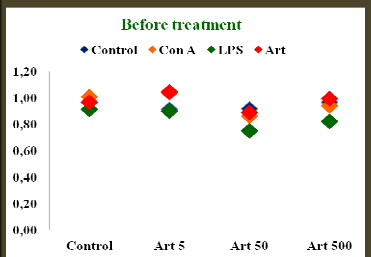
The level of serum gamma-globulin was significantly higher in chickens in-feed treated with 50 and 500 ppm artemisinin than in untreated chickens. Generally, the weight of lymphoid organs was lower in experimental groups comparing with control group, but only in case of bursa the difference was statistic significantly. Interestingly, the weight of bursa and spleen was higher in chickens treated with 5 ppm artemisinin than in untreated chickens.

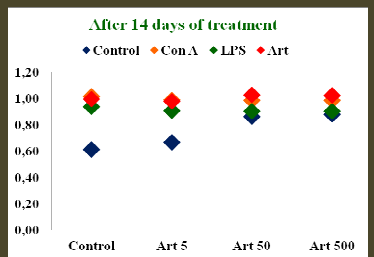


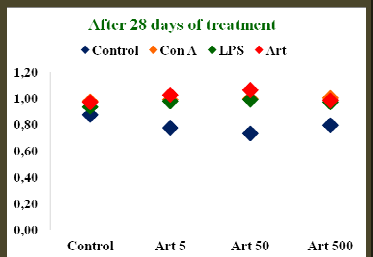


Lymphocyte proliferation assay

The stimulation index of lymphocytes was higer in chickens treated with 50, and 500 ppm artemisinin and *in vitro* stimulated with concavalin A and lipopolysaccharide, but lower in case of *in vitro* stimulation with artemisinin. Over time, stimulation index decreased in all treated chickens, but greater in chickens treated with 50 ppm artemisinin, and *in vitro* stimulated with concavalin A and artemisinin, while in case of lipopolysaccharide increased.

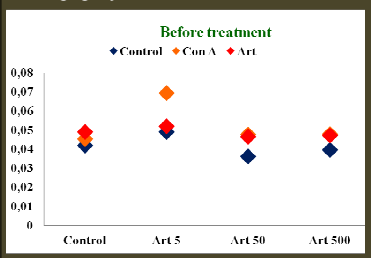


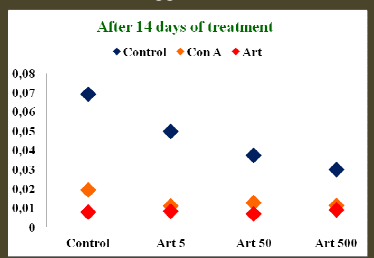


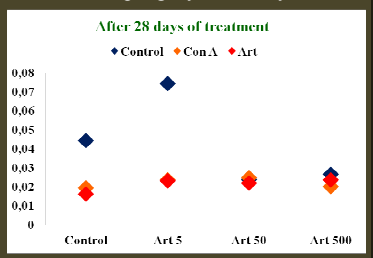


Phagocytosis assay

The *in vitro phagocytic activity was supressed by* artemisinin only at 28 days after in-feed treatment with artemisinin, but without statistical significance comparing with control group. Interestingly, in variants without stimulation, and stimulation with lipopolysaccharide, in-feed treatment with 50, and 500 ppm artemisinin stimulated the *in vitro* phagocytic activity.







CONCLUSIONS

Artemisinin is relatively immunotolerant in broiler chickens, and further investigations are needed to make a final conclusion.

Acknowledgements. This work was supported by UEFISCDI, project number PN II-PCCA Type 2 110/2012.

EFFECT OF ARTEMISININ ON IMMUNE SYSTEM IN CHICKENS: PRELIMINARY RESULTS

Adriana Györke[1]*, Loredana Pop[1], Anamaria Paştiu[1], Zsuzsa Kalmar[1], Mirabela Dumitrache[1], Flaviu Tăbăran[2], Mircea Mircean[3], Cristian Magdaş[1], Viorica Mircean[1], Vasile Cozma[1]

University of Agricultural Science and Veterinary Medicine Cluj-Napoca, Faculty of Veterinary Medicine, 3-5 Calea Mănăştur, 400372 Cluj-Napoca, Romania
[1]Parasitology and Parasitic Diseases Department ; [2]Pathologic Anatomy and Necropsy; [3]Internal Medicine Department
***titilincua@yahoo.com**

INTRODUCTION

Artemisinin is a sesquiterpene lactone isolated from the Chinese herb *Artemisia annua*, and has been used for centuries to treat malaria. This compound has antiviral (Romero et al., 2006; Efferth et al., 2008), anti-cancer effects (Efferth, 2006) and antibacterial properties (Dhingra et al., 2000). Artemisinin was found to be effective in controlling poultry coccidiosis (Arab et al., 2006; Naidoo et al., 2008; del Cacho et al., 2010), and it is also efficient on *Schistosoma, Babesia caballi*, hepatic trematodes, trypanosomiasis and leishmaniosis (Utzinger, 2007; Kumar et. al., 2003; Keiser et. al., 2006; Mishina et al., 2007; Sen et al., 2010).

Artemisinin contains an endoperoxide bridge which by reductive breaking, operated by iron complexes, generates reactive radicals that would produce damage to the parasites (Galasso et al., 2007). It was also shown that treatment with artemisinin alters the process of oocysts wall formation in case of *Eimeria* (del Cacho et al., 2010). Although artemisinin has been proven to be safe in therapeutic doses, some researchers have shown side effects of this compound. Embryolethality, morphological abnormalities during early pregnancy, hormonal imbalances were induced by treatment with artemisinin in mammals (Boareto et al., 2008). In broiler chickens, artemisinin causes decreases in red blood cells count and hematocrit, anemia, kidney, liver and brain lesions (Arab et. al., 2009; Shahbazfar et. al, 2011). Effect on immune system was studied in a mouse model. The study showed that topical administration of artemisinin could supress CHS response. Artemisinin had an inhibitory effect on IL-17 production, diminished the level of IL-6 and enhanced TGF-β (Li et al., 2012).

The present study aimed to determine the effects of different doses of artemisinin on the immune system of broiler chickens following chronic oral intake.

REFERENCES

1. **Arab, H.A., Rahbari, S., Rassouli, A., Moslemi, M.H., Khosravirad, F.** Determination of artemisinin in *Artemisia sieberi* and anticoccidial effects of the plant extract in broiler chickens. *Trop Anim Health Prod*. 2006, 38, 497-503.
2. **Arab HA, Mardjanmehr SH, Shahbazfar A, Rassouli A, Abdollahi M, Nekouie O.** Toxicopathologic Effects of Artemisinin in Broiler Chickens Following a Single Oral Dose: An LD 50 Study. *Int J Poultry Sci*. 2009;8:808–812.
3. **Boareto A.C., Muller J.C., Bufalo A.C., Botelho G.G.K., de Araujo S. L., Foglio M.A., de Morais R. N., Dalsenter P.R.** Toxicity of artemisinin [Artemisia annua L.] in two different periods of pregnancy in Wistar rats, *Reprod. Toxicol.* 2008; 25,239–246
4. **del Cacho E., Gallego M., Francesch M., Quílez J., Sánchez-Acedo C.** (2010). Effect of artemisinin on oocyst wall formation and sporulation during *Eimeria tenella* infection. *Parasitol Int*. 2010, 59, 506-511.
5. **Dhingra, V., Pakki, S.R. & Narasu, M.L.** Antimicrobial activity of artemisinin and its precursors. *Curr Sci*. 2000, 78, 709-713;
6. **Efferth T.** Molecular pharmacology and pharmacogenomics of artemisinin and its derivatives in cancer cells. *Curr Drug Targets* 2006;7:407-21.
7. **Efferth, T., Romero M. R., Wolf , D. G., Stamminger, T., Marin, J. J. and Marschall, M.** The antiviral activities of artemisinin and artesunate. *Clin. Infect. Dis.* 2008, 47:804–811.
8. **Galasso V., Kovac B., Modelli A.** A theoretical and experimental study on the molecular and electronic structures of artemisinin and related drug molecules. *Chem phys*. 2007, 335,141–154.
9. **Keiser, J.; Utzinger, J.** Food-borne trematodiasis: current chemotherapy and advances with artemisinins and synthetic trioxolanes. *Trends Parasitol*. 2007, v.23, n.11, p.555-562;
10. **Kumar, S.; Gupta, A.K.; Pal, Y.; Dwivedi, S.K.** In vivo therapeutic efficacy trial with artemisinin derivative, Buparvaquone and Imidocarb Dipropionate against *Babesia equi* infection in donkeys. *J Vet Med Sci*. 2003, v.65, n. 11, p.1171-1177;
11. **Li T., Chen H., Wei N., Mei X., Zhang S., Liu DL., Gao Y., Bai SF., Liu XG., Zhou YX.** Anti-inflammatory and immunomodulatory mechanisms of artemisinin on contact hypersensitivity. Int Immunopharmacol. 2012; 12(1):144-50
12. **Mishina, Y.V., Krishna, S., Haynes, R.K., Meade, J.C.** Artemisinins Inhibit Trypanosoma cruzi and Trypanosoma brucei rhodesiense In Vitro Growth. Antimicrob. Agents Chemother. 2007, 51:1852–1854.
13. **Naidoo, V., McGaw, L.J., Bisschop, S.P., Duncan, N., Eloff, J.N.** The value of plant extracts with antioxidant activity in attenuating coccidiosis in broiler chickens. Vet Parasitol. 2008, 153, 214-219.
14. **Romero MR, Serrano MA, Vallejo M, Efferth, T, Alvarez M, Marin JJG.** Antiviral effect of artemisinin from *Artemisia annua* against a model member of the *Flaviviridae* family, the bovine viral diarrhoea virus (BVDV). *Planta Med* 2006;72:1169-74.
15. **Sen R., Ganguly S., Saha P.,Chatterjee M.** Efficacy of artemisinin in experimental visceral leishmaniasis. *Int J Antimicrob Ag*. 2010, 36, 43–49
16. **Shahbazfar, A.A., Mardjanmehr, S.H., Arab, H.A., Rassouli, A., Abdollahi, M.** Effects of artemisinin in broiler chickens following chronic oral intake. *Trop. Anim. Health Prod*. 2011, 43, 843-849.
17. **Utzinger J, Xiao SH, Tanner M, Keiser J.** Artemisinins for schistosomiasis and beyond. *Curr Opin Investig Drugs*, 2007;8:105-16.

MATERIAL AND METHODS

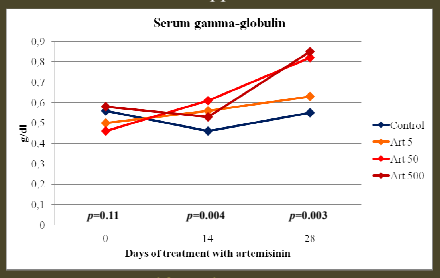
We used four experimental groups of 10 chickens (ROSS 308), divided in two replicates of five chickens (one females, and one males). One group received no treatment (control group) and three groups received artemisinin in their diet at 5, 50 and 500 ppm concentrations. The treatment started when chickens were 18 days and lasted for 28 days (46 days old). Blood and serum samples were harvested from each chicken before treatment and two times after treatment at 14 and 28 days. Finally, chickens were euthanized by cervical dislocation and lymphoid organs (bursa of Fabricius, spleen, and thymus) collected. Feed and water were provided *ad libitum*, and the lighting was continuous. The chickens were reared in cages. Artemisinin of 98% purity was purchased from INTATRADE Chemicals GmbH, Germany.

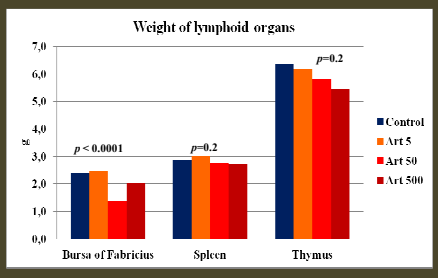
The effect of artemisinin on immune system in broiler chickens was assessed by lymphocyte proliferation assay using whole blood (mitogens: concavalin A, lipopolysaccharide, artemisinin), phagocytosis assay, evaluation of serum gamma-globulin levels, and development of lymphoid organs (weight).

RESULTS

Serum gamma-globulin levels and weight of lymphoid organs

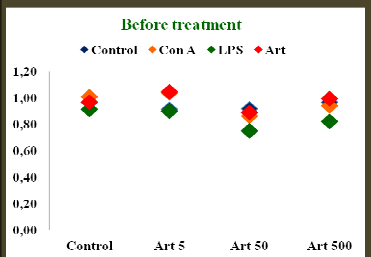
The level of serum gamma-globulin was significantly higher in chickens in-feed treated with 50 and 500 ppm artemisinin than in untreated chickens. Generally, the weight of lymphoid organs was lower in experimental groups comparing with control group, but only in case of bursa the difference was statistic significantly. Interestingly, the weight of bursa and spleen was higher in chickens treated with 5 ppm artemisinin than in untreated chickens.

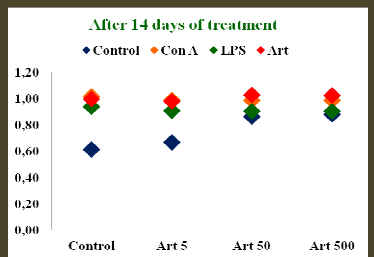


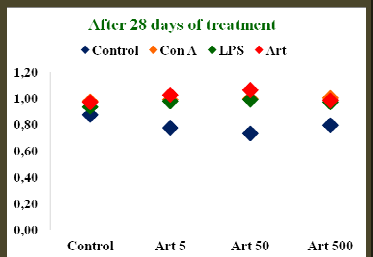


Lymphocyte proliferation assay

The stimulation index of lymphocytes was higer in chickens treated with 50, and 500 ppm artemisinin and *in vitro* stimulated with concavalin A and lipopolysaccharide, but lower in case of *in vitro* stimulation with artemisinin. Over time, stimulation index decreased in all treated chickens, but greater in chickens treated with 50 ppm artemisinin, and *in vitro* stimulated with concavalin A and artemisinin, while in case of lipopolysaccharide increased.

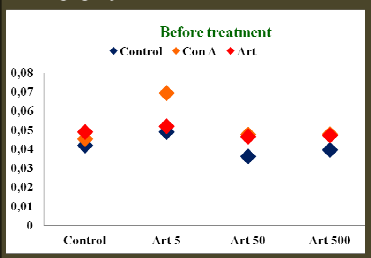


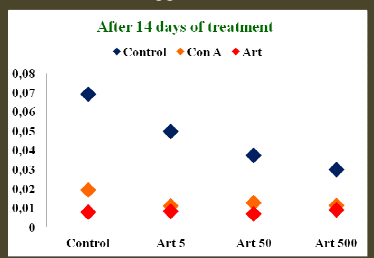


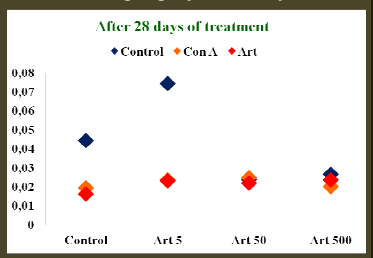


Phagocytosis assay

The *in vitro phagocytic activity was supressed by* artemisinin only at 28 days after in-feed treatment with artemisinin, but without statistical significance comparing with control group. Interestingly, in variants without stimulation, and stimulation with lipopolysaccharide, in-feed treatment with 50, and 500 ppm artemisinin stimulated the *in vitro* phagocytic activity.







CONCLUSIONS

Artemisinin is relatively immunotolerant in broiler chickens, and further investigations are needed to make a final conclusion.

Acknowledgements. This work was supported by UEFISCDI, project number PN II-PCCA Type 2 110/2012.