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Production of lambs' resilience to *Haemonchus contortus*

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ABSTRACT

Haemonchus (H.) contortus infection in sheep is a major health problem in tropical and sub-tropical regions that cause great economic losses. Our previous studies have shown that improved uterine environments during pregnancy can improve postnatal growth and health performance of the offspring, indicated by lower mortality and morbidity. In the present experiment, we evaluated the resistance and resilience to *H. contortus* of lambs born to ewes injected with pregnant mare's serum gonadotropin (PMSG) prior to mating. Improvement of the uterine environment was conducted by increasing endogenous secretion of estrogen and progesterone as pregnancy hormones during pregnancy by injecting the ewes with PMSG prior to mating. A total of 16 lambs, regardless of sex, at the age of 5 months were assigned into a 2 × 2 factorial experiment with 4 replications. The first factor was PMSG injection, consisting of two levels, i.e., lambs born to ewes without PMSG injection (Non-PMSG lambs) and those born to PMSG-injected ewes (PMSG lambs). The second factor was the infection of lambs with *H. contortus* at the age of 5 months, consisting of two levels, i.e., lambs without infection (Non-infected lambs) and lambs infected with *H. contortus* (Infected lambs). Non-infected lambs were administered with distilled water in a capsule without infective larvae. Infected lambs were individually infected with a single dose containing 1200 L3 of *H. contortus*. Compared to non-PMSG lambs, PMSG lambs tended to have better prenatal growth indicated by greater birth weights (P = 0.06). The improved prenatal growth during pregnancy improved the postnatal growth and health performance of the lambs. Three months after infection of *H. contortus*, non-PMSG lambs and PMSG lambs had similar worm counts. However, the PMSG lambs showed significantly higher resilience to *H. contortus* as indicated by the lower fecal egg counts 6–10 weeks after infection. The higher resilience of the PMSG lambs was shown by the positive growth rate during infection, while non-PMSG lambs had a negative growth rate after infection. Prior to infection, PMSG lambs showed a higher segmented neutrophil percentage with lower lymphocyte numbers. Three months after infection, PMSG lambs had significantly higher lymphocyte and thrombocyte numbers as well as mean corpuscular hemoglobin concentration (MCHC) with lower neutrophil and monocyte numbers. The conclusion of this study is that the improvement of the uterine environment during pregnancy could be used to produce superior offspring with high resilience to the infection of *H. contortus*.

1. Introduction

Parasite infestation by nematode in the intestine is a common problem in the animal husbandry industry. The infection of this parasite increases the operation costs and causes economic losses (Sackett et al., 2006). In addition, the problem is worse with the report that some intestinal parasites have increased resistance due to inappropriate and uncontrolled use of anthelmintics (Luffau et al., 1990). One of the main gastrointestinal parasites in sheep is the nematode worm of *Haemonchus (H.) contortus*. *H. contortus* infection causes decreased body weight and anemia (Strain and Stear, 2001). In the case of chronic infection, the decreases in body weight and hematocrit levels are not normally found as compared to the non-infected animals. In hyper-acute cases, the infected animals can die quickly due to the serious bleeding in the gastrointestinal tract (Roberts and Swan, 1982).

Several experiments have been conducted to map factors increasing

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the resistances of animals to *H. contortus*. One of the factors that has become a popular topic in veterinary parasitology is the genes that control the resistance phenotype (Alba-Hurtado and Munoz-Guzman, 2013). However, it is known that the genetically resistance mother does not always produce resistant offspring because the inheritance of the resistance phenotype to the offspring is not absolute (Marshall et al., 2013). Therefore, crossing with a strict selection can be used to produce offspring having high resistance. However, the method takes longer and requires strict record keeping (Wanyangu et al., 1997). Therefore, a more efficient new approach or method is required to optimize and increase the expression of the resistance genotype in offspring.

Based on the understanding that the resistance phenotype is a form of united and coordinated function contributed by some bodily functions, to optimize and activate the resistance phenotypes, the improvement of all body systems is required. One approach is to optimize the development of the organ systems during the growth and development of the embryo and fetus during pregnancy. Improvement of growth and development of the embryo and fetus during pregnancy can be achieved by increasing the signals and factors controlling the growth and development of the uterine and placenta (Fowden et al., 2008; Fowden and Forhead, 2009a) by optimizing the availabilities of pregnancy hormones during pregnancy. Improved uterine and placental structure and functions during pregnancy improve gene expression and protein abundance that eventually function as epigenetic signals that affect fetal growth and development as well as phenotype diversity, which affects postnatal growth and physiological variability (Fowden and Forhead, 2009b).

Maternal serum progesterone concentrations, as the main pregnancy hormones, can be increased by gonadotropin injection of the mothers prior to mating that eventually improves prenatal and postnatal growth in sheep and swine (Manalu et al., 2000; Manalu and Sumaryadi, 1998; Rayer et al., 2015a, b; Mege et al., 2006). The improved postnatal growth also associates with the increased survival as indicated by the lower morbidity and mortality (Rayer et al., 2015a, b; Mege et al., 2006). Based on these phenomena, the present experiment was designed to evaluate the resistance and resilience to *H. contortus* of lambs born to ewes injected with pregnant mare's serum gonadotropin (PMSG) prior to mating. PMSG injection prior to mating increases endogenous secretion of pregnancy hormones that improve the uterine environment during pregnancy. Our previous studies have shown that PMSG-injected does prior to mating had greater uterus morphometric and histometric parameters that correlated well with the fetal weight at the end of embryonic stage of pregnancy (Arif et al., 2018). This is the first report on the improvement of lambs' resistance and resilience to *H. contortus* by injecting the mother with PMSG prior to mating to improve the growth and development of the uterus and placenta that will support the prenatal growth of the offspring during pregnancy.

2. Materials and methods

2.1. Experimental design

An experiment was designed in a completely randomized fashion with 2 × 2 factorial arrangement, and each experimental unit used 4 lambs. The experiment used 8 lambs born to PMSG-injected ewes (PMSG lambs) and 8 lambs born to non-PMSG injected ewes (non-PMSG lambs). The age of the experimental lambs at the beginning of experiment was 5 months, and there was no sexual grouping of the experimental lambs. The first factor was the PMSG injection, consisting of two levels, i.e., lambs born to ewes without PMSG injection prior to mating (non-PMSG lambs) and lambs born to PMSG-injected ewes prior to mating to improve endogenous secretions of pregnancy hormones to optimize the uterine environment during pregnancy (PMSG lambs). The second factor was the infection of the experimental lambs with *H. contortus,* consisting of two levels, i.e., lambs without infection *H. contortus* as a control (non-infected lambs) and lambs infected with *H. contortus* (infected lambs). The lambs in the infected lambs group were individually infected with a single dose containing 1200 L3 of *H. contortus* (modified from Ginting et al., 1999), while the non-infected group was administered with distilled water in a capsule without *H. contortus*. The experiment was conducted according to the National Institute of Health's guide for the Care and Use of Laboratory animals (NIH Publications No. 8023, revised 1978).

2.2. Experimental animals

To produce lambs used in the experiment, all maternal ewes were injected with prostaglandin F2 alpha ($PGF_{2\alpha}$) two times with an 11-day interval to synchronize the estrus cycle prior to mating. The PMSG-injected ewes were injected with PMSG at a dose of 7.5 IU/kg body weight (BW) at the same time as the second $PGF_{2\alpha}$ injection (Andriyanto et al., 2017). The non-PMSG-injected ewes were injected with 0.7% NaCl solution. At the estrus period, all experimental ewes were mated naturally by mixing with selected male sheep for 1 week. The experimental ewes were maintained until parturition. At parturition, the birth weights of the lambs were measured. At weaning time at the age of 20 weeks postpartum, the lambs were selected for infection of infective *H. contortus* larvae. Prior to infection, the experimental lambs were raised intensively in cages with the maternal ewes. During the treatment, the experimental lambs were maintained intensively in cages.

2.3. Production of infective H. Contortus larvae, artificial infection, and collecting samples

Two donor sheep were prepared for production of infective *H. contortus* larvae. The donor sheep were administered anthelmintics for 3 consecutive days. The number of eggs in each gram of feces was measured, and the sheep were free of worm infection. The donor sheep were acclimatized for 1 week. The donor sheep were fed with grass and non-grass forage feed free of worm eggs and larvae.

The mature female *H. contortus* worms were collected from the abomasum of sheep from the local slaughterhouse. The mature worms were collected, grinded to obtain eggs, and then cultured and nourished. After 1 week of culturing, the collection of L3 larvae was conducted. The collected L3 larvae were put into a soft capsule for further use to infect the donor experimental sheep. Two weeks after infection, the number of eggs per gram of feces was counted to ensure that the donor sheep were infected with *H. contortus,* and the eggs were found in the feces. The feces of the donor sheep was cultured for further collection of L3. The dose of L3 infection was estimated by calculating the concentrations, i.e., the number of L3 larvae per milliliter of distilled water. The number of infected L3 larvae was 1200 L3 larvae per experimental lamb (modified from Ginting et al. (1999).

2.4. Infection of experimental lambs

The experimental lambs were infected with infective larvae packed in a capsule directly into the stomach using a stomach tube. The uninfected lambs were administered with distilled water in a capsule without infective larvae. After infection, the fecal samples were collected weekly up to 13 weeks post-infection to measure the number of worm eggs in the feces or fecal egg count (FEC). Thirteen weeks after infection, the fecal samples were collected for 24 h to measure the total worm eggs to calculate female worm fecundity. At the end of experiment (13 weeks post infection), the blood samples were also collected to measure blood cell parameters (erythrocyte, leukocytes, and thrombocytes). After collecting blood samples, the infected lambs (non-PMSG lambs and PMSG lambs) were sacrificed to obtain the abomasum for measurement of the number of male and female worms, total number of male and female worms, and the length of male and female worms in the mucosa of the abomasum. Since the non-infected lambs