

The effect of garlic extract on growth, haematology and cell-mediated immune response of newborn goat kids

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Abstract

The present study was carried out to evaluate the effect of different levels of garlic extract supplemented in milk on growth rate, haematology and cell-mediated immune response of Markhoz newborn goat kids. Twenty four newborn goat kids (aged 7±3 days) were randomly assigned to four groups. The groups consisted of control (received milk without garlic extract), T1, T2 and T3 which received milk supplemented with 62.5, 125 and 250 mg aqueous garlic extract per kg live weight per day for 42 days, respectively. Body weights were measured weekly throughout the experimental period. At day 42, about 10 ml blood samples were collected from each kid via the jugular vein for haematological study. Cell-mediated immune response was evaluated through double skin thickness after intradermal injection of phyto-hematogglutinin (PHA) at day 21 and 42. Total gain was significantly higher for kids in T3 ($P < 0.05$) compared with the control group. Average daily gain (ADG) in T3 group in week 4–5 was higher ($P < 0.05$). Significant differences in globulin ($P < 0.01$), hemoglobin (Hb; $P < 0.001$), hematocrit (PCV; $P < 0.001$), erythrocyte (RBC; $P < 0.001$), neutrophil ($P < 0.001$), lymphocyte ($P < 0.001$) and leukocyte (WBC; $P < 0.001$) were observed among groups. Hb, PCV, RBC, lymphocytes and WBC were higher in kids given garlic extract supplementation. There was a significant difference of double skin thickness among the groups at day 42 ($P < 0.01$). In conclusion, this study indicated that milk supplemented with aqueous garlic extract improved growth rate and immunity of newborn goat kids.

Keywords: garlic extract, growth rate, haematology, cell-mediate immune response, newborn goat kids

1 Introduction

Survival of newborn-kids is an important determinant of profitability in a goat farm operation (Shokrollahi *et al.*, 2013). Newborns are exposed to a variety of environmental and physiological stress. Daniels *et al.* (2000) reported that mortality rates varied between 15 and 51 % with rates as high as 35 % supposed acceptable among large sheep farms. Losses approach-

ing 100 % have been observed in farms experiencing acute diseases (Shelton & Willingham, 2002). To prevent such disasters, it is critical to improve the immune system of newborn kids, particularly in the first few weeks of life in order to combat a variety of environmental and physiological stressors. For example, the rate of mortality for Markhoz breed from birth to weaning is around 10 % (Rashidi *et al.*, 2011). Present immunisation practices focus on immunising the pregnant goats before parturition to get the best possible build-up of antibodies and passive immunity to the kids via colostrum. Although this passive immunity works well to protect most kids against common infectious agents for the first ten to twelve weeks of life, it depends on

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the strength of the goat's immune system. Therefore, there can be a high degree of variability in the quality of passive immunity acquired by the kids. Antibiotic and growth promoters have been extensively used in animal industry worldwide for decades to prevent diseases and metabolic disorders. In 2006 the European Union has banned the use of antibiotics as growth and immune promoters due to concerns over the transfer of bacterial resistance to antibiotics to humans (Windisch *et al.*, 2008). Nowadays, the use of phytochemical compounds such as herbs with broad antioxidative (Wei & Shibamoto, 2007), and antimicrobial effects (Özer *et al.*, 2007), as well as growth and immune boosters (Chen *et al.*, 2008) have received much attention as most favourable alternatives to antibiotics for animal industry. Garlic (*Allium sativum*) has been used as medicinal herb since time immemorial in almost every known civilisation (Rivlin, 2001). The exclusive flavour and immune-boosting function of garlic is generally attributed to its rich content of sulphur containing substances, i.e. alliin, γ -glutamylcysteine, and their derivatives (Amagase *et al.*, 2001; Tsai *et al.*, 2012) which are converted into thiosulphinates via enzymatic reactions when raw garlic is processed (Amagase, 2006). Numerous studies determined garlic and its bioactive sulphur compounds to be potent antioxidants by displaying radical-scavenging activity and modulating cellular antioxidant enzyme activity (Tsai *et al.*, 2012). Garlic has immune raising activities that involves promotion of lymphocyte formation, cytokines release, phagocytosis and natural killer cells activity (Kyo *et al.*, 1998). Other properties of garlic are antibacterial and antifungal (Ankri & Mirelman, 1999; Sivam, 2001), antiparasitic, anthelmintic (Worku *et al.*, 2009), antiviral, antithrombotic, vasodilatory and anticancer (Agarwal, 1996; Tsai *et al.*, 2012). Furthermore, garlic or allicin supplementation exerted positive effect on newborn animals mainly because of its immunity improvement effect (Tatara *et al.*, 2005; Wang *et al.*, 2011).

According to the latest livestock census, conducted in 2014, there are more than 22 million heads of goats in Iran (FAO, 2016). Iranian goats are favoured in most rural areas of Iran and mainly reared in traditional systems by small holders (Vahidi *et al.*, 2014). Markhoz goat is one the most important indigenous breed raised in Kurdistan region. Newborn kids are allowed to remain with their dam for a week, before being separated and suckled twice a day until weaning (4 months of age). However, no known study is available on the potential growth promoting effects of garlic extract when added to milk on growth and immunity of newborn goat kids.

Therefore, the present study was undertaken to investigate the effect of garlic extract at different supplemental levels on growth rate, haematology and cell-mediated immune response in newborn goat kids.

2 Materials and methods

2.1 Animal management and experimental design

This experiment was carried out at the Markhoz Goat Research Station of Sanandaj in the Kurdistan province, Iran. A total of twenty-four newborn male Markhoz goat kids (about 7 ± 3 days of age) were randomly allotted to four treatment groups (Control, T1, T2 and T3). Groups were fed with enriched milk with 0 (control group), 62.5 (group T1), 125 (group T2) and 250 (group T3) mg aqueous garlic extract per kg of live body weight per day for 42 days. These animals were selected according to the parity of dams (all dams were homogeneous for parity), weight and nutrition during their pregnancy. All kids were fed colostrum on the first days after their birth. Thirty ml water containing the required amount of garlic extract in each group was mixed with 70 ml milk obtained from each mother. The quantity of garlic extract was adjusted once per week according to the body weight. Milk was hand-milked from the mothers, labelled and mixed with the assigned amount of aqueous garlic extract.

Kids in all experimental groups were managed similarly. Health status of kids was monitored and kids which encountered pneumonia and/or diarrhea were treated or deleted before the start of the experiment. Each kid was kept with its mother (one kid per dam) in separate pens equipped with feed and water throughout the experiment. Before feeding the supplemented milk, kids were separated from their mothers (08:00 am) and fasted for 3 h. Then they were fed garlic extract supplemented milk using pacifiers (11:00 am).

2.2 Weighing and sampling

Kids were weighed weekly from the beginning to the end of the experiment (6 weeks). About 10 ml blood samples were collected from each kid through the jugular vein at the end of the study (day 42). Two and half ml of blood anti-coagulated with EDTA were used for blood cells counting and 7.5 ml transferred to a plane tube for serum separation. All tubes were instantly kept at 4 °C and then centrifuged ($3,000 \times g$ for 10 min), the obtained serum was separated and all samples were transferred to the laboratory and stored at

–20 °C until analyses. Anticoagulated blood was analysed after collection for measurements of haematocrit (PCV), haemoglobin (Hb) and leukocyte and erythrocyte counts (WBC and RBC) by micro-haematocrit, cyanmethaemoglobin and standard manual methods, respectively. Differential leukocyte counts were performed on routinely prepared Giemsa-stained blood films using the cross-sectional technique (Jain, 1986). Serum globulin was measured using a commercial kit (Pars Azmun R2 11005). Average daily gain (ADG) was calculated weekly throughout the experimental period.

2.3 Skin testing

Cell-mediated immune response was evaluated through determining double skin thickness in response to phytohaemagglutinin (PHA) using the test procedure reported by Lacetera *et al.* (1999). Skin tests were performed on days 21 and 42 after the beginning of the experiment. To this end, 250 µg PHA diluted in 0.1 ml phosphate buffer saline (PBS) was intradermally injected to a shaved area on the right lumbar back using an automatic injector. Double skin thickness was measured using a digital calliper before (time 0) and 8, 16, and 24 h after injection of PHA. Sterile PBS was injected approximately 10 cm from the injection site of PHA to test of any skin responses to PBS alone.

2.4 Statistical analysis

Data for haematology parameters, ADG and weights were analysed according to a completely randomized design using the General Linear Models of SAS (version 9.2). Double skin thickness data were submitted to the MIXED procedure, considering the skin thickness before PHA injection (time 0) as covariate. Means were separated by LSD, and least squares means and SEM for all data are presented. Main effects were discussed if $P < 0.05$.

3 Results

3.1 Body weight gain

The initial body weight and weekly body weight after garlic extract supplementation of the kids showed no significant differences among groups (Table 1). Total gain was significantly higher in T3 ($P < 0.05$) compared with the other groups but there were no significant differences among control, T1 and T2 groups although total gain tend to be higher in the treatment groups compared to the control animals (Table 1). ADG of T3 kids was significantly higher in week 4–5 ($P < 0.05$) but showed no difference in other weeks compared with other groups. Overall ADG was lower for the control than those in the other groups (Table 1).

Table 1: Mean of live weights (kg), ADGs (g) and total gain (kg) in different treatment groups ($n=6$ per group) for Markhoz newborn goat kids

Trait	Control	T1	T2	T3	P-value
LWW1	4.6±0.31	4.8±0.10	4.2±0.20	4.7±0.20	NS
LWW2	5.2±0.35	5.3±0.14	5.1±0.25	5.5±0.19	NS
LWW3	5.7±0.35	5.9±0.13	5.6±0.26	6.1±0.21	NS
LWW4	5.9±0.35	6.2±0.20	6.2±0.41	6.7±0.27	NS
LWW5	6.2±0.33	6.8±0.26	6.9±0.55	7.8±0.43	NS
LWW6	7.0±0.35	7.5±0.33	7.7±0.72	8.8±0.57	NS
ADG1-2	83.3±17.07	83.3±15.39	128.5±28.33	119.0±17.56	NS
ADG2-3	57.1±5.21	66.7±17.93	71.4±16.07	76.2±12.59	NS
ADG3-4	50.0±13.67	47.6±11.47	83.3±27.94	104.7±15.50	NS
ADG4-5	66.7±15.04 ^b	75.0±6.32 ^{ab}	97.6±24.30 ^{ab}	154.7±30.28 ^a	*
ADG5-6	86.9±15.67	98.8±19.73	145.24±28.67	133.4±26.49	NS
Overall ADG (g)	57.3±6.38	63.4±9.67	83.7±17.02	98.0±10.08	NS
Total gain (kg)	2.4±0.27 ^b	2.7±0.41 ^{ab}	3.5±0.71 ^{ab}	4.1±0.42 ^a	**

LWW1: Live weight week 1; LWW2: Live weight week 2; etc.

ADG1-2: Average Daily Gain between weeks 1 and 2 ; ADG 2-3: Average Daily Gain between week 2 and 3; etc.

NS = $P > 0.05$; * = $P < 0.05$; ** = $P < 0.01$; Means in same row with different letter are significantly different.

The Control, T1, T2 and T3 groups were supplemented with 70 cc of milk and 0, 62.5, 125 or 250 mg of garlic extract per kg of live body weight per day dissolved in 30 ml water.

3.2 Haematology and blood parameters

The effects of garlic supplementation on haematology and blood parameters of newborn goat kids are shown in Table 2. Significant differences in globulin ($P < 0.01$), Hb ($P < 0.001$), PCV ($P < 0.001$), RBC ($P < 0.001$), neutrophil ($P < 0.001$), lymphocyte ($P < 0.001$) and WBC ($P < 0.001$) were observed among groups. Hb, PCV, RBC, lymphocytes and WBC were higher in kids given garlic extract supplementation compared to those in the control group with group T2 showing the highest levels, while neutrophil levels significantly decreased in garlic supplemented groups.

3.3 Cell-mediate immune response

There were no differences among the groups in terms of skin thickness at PBS injection sites and therefore these were not used to adjust PHA-induced skin thickness. However, there were significant differences in increase of skin thickness after injection of PHA among groups at day 42 (Fig. 1b) ($P < 0.01$) but not at day 21 (Fig. 1a). Skin thickness increased strongly after injection at 0 h for all four groups but there was no significant difference among the groups at 0, 8, 16 and 24 h (Fig. 1a and 1b).

Table 2: Mean concentration of different blood biochemical and haematology factors in different groups for Markhoz newborn goat kids

Factor	Control	T1	T2	T3	P-value
Globulin (g/dl)	3.10±0.16 ^a	3.20±0.12 ^a	2.55±0.01 ^b	2.77±0.06 ^{ab}	**
Hb (g/dl)	7.22±0.16 ^b	9.10±0.29 ^a	10.02±0.29 ^a	9.45±0.29 ^a	***
PCV (%)	15.37±1.54 ^b	26.62±1.91 ^a	31.52±0.98 ^a	26.80±0.86 ^a	***
RBC (×106/μl)	9.69±0.91 ^b	16.01±0.80 ^a	17.97±0.47 ^a	15.65±0.36 ^a	***
Neutrophiles (%)	43.25±1.95 ^a	37.00±1.65 ^b	34.25±1.078 ^b	35.00±0.86 ^b	***
Lymphocyte (%)	57.00±1.63 ^b	62.75±1.82 ^a	64.50±1.25 ^a	63.75±0.96 ^a	***
WBC (×106/μl)	17.05±1.09 ^b	14.25±1.21 ^b	22.54±1.04 ^a	15.25±0.788 ^b	***

** = $P < 0.01$; *** = $P < 0.001$; Means in same row with different lowercase letter are significantly different.

The Control, T1, T2 and T3 groups were supplemented with 70 cc of milk and 0, 62.5, 125 or 250 mg of garlic extract per kg of live body weight per day dissolved in 30 ml water.

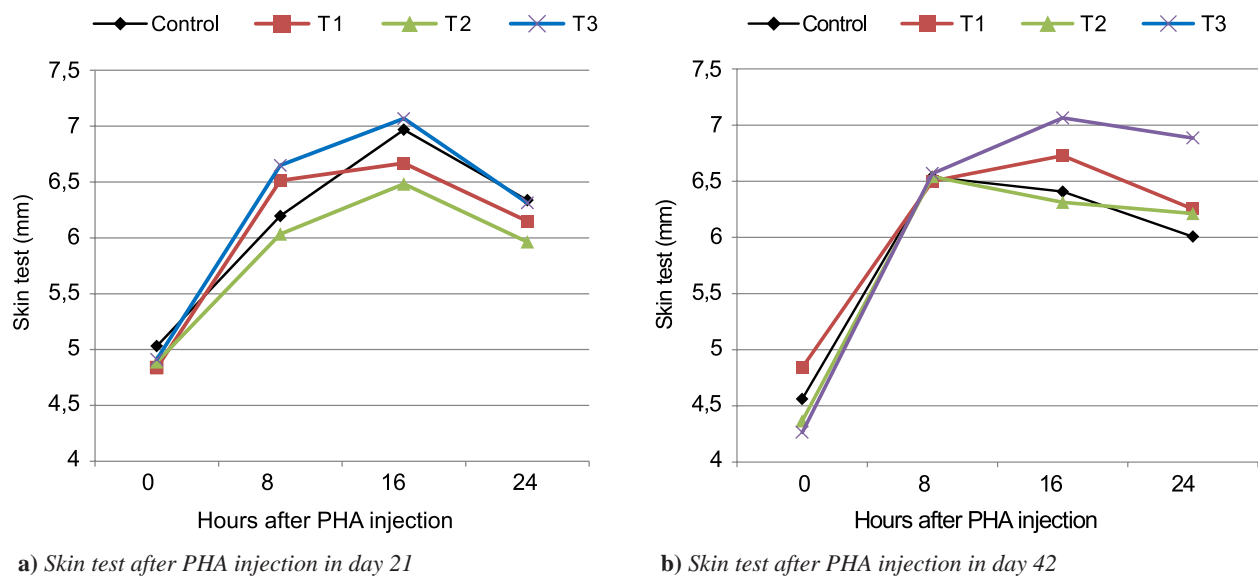


Fig. 1: Double skin thickness of Markhoz newborn goat kids in different treatments (0 (control), 62.5 (T1), 125 (T2), and 250 (T3) mg aqueous garlic extract per kg of live body weight per day) at 0, 8, 16, and hours after PHA injection in day 21 (Fig. 1a) and in day 42 (Fig. 1b).

4 Discussion

Many studies have reported that garlic can improve the gastrointestinal status of mammalian species by avoiding the proliferation of pathogens and bacteria thereby helping to maintain health conditions and subsequently improve growth performance (Ankri & Mirelman, 1999; Ross *et al.*, 2001; Rao *et al.*, 2003). The results of the current study showed that milk supplementation with aqueous garlic extract improved total gain in goat kids as kids receiving higher levels of garlic extract showed higher weight and overall ADG during the experiment (Table 1). It has been reported that garlic supplementation led to improved body weight and growth in piglets (Kleczkowski *et al.*, 2004; Grella & Klebaniuk, 2007; Tatara *et al.*, 2005) and lambs (Badias & Yaniz, 2004). Moreover, Yan *et al.* (2011) found that ADG of pigs fed diets including fermented garlic powder was higher compared with control group. In addition, significant increase in daily gain by adding of 2.5% natural juice containing garlic have been reported for growing buffalo calves (Ahmed *et al.*, 2009). Tatara *et al.* (2008) also suggested that aged garlic extract or allicin increased the final body weight of piglets exposed to early weaning. In another study, significant effect of supplementation of garlic extract on body weight of crossbred dairy calves has been reported by Ghosh *et al.* (2010). These authors stated that this result may be due to the antimicrobial action of garlic and consequently improved enteric health, proper maintenance of liver function, enhancement of the activity of pancreatic lipase and amylase, and reduction in crypt depth in the ileum. In contrast with the result of the current study, previous studies on pig (Chen *et al.*, 2008; Horton *et al.*, 1991; Langendijk *et al.*, 2007) and chicken (Ao *et al.*, 2011; Freitas *et al.*, 2001; Konjufca *et al.*, 1997; Thanikachalam *et al.*, 2010), reported no positive effects of garlic supplementation on growth performance. Likewise, diet supplementation with 30 and 60 kg garlic bulbs per ton feed showed no effect on growth performance in growing lambs (Bampidis *et al.*, 2005). The inconsistent results may be due to differences in the type, quality, or quantity of the supplemented garlic and also species and age of the animals.

Globulin levels are undoubtedly important for sustaining a healthy immune system and immune functions in the blood (Shokrollahi *et al.*, 2015). Our results showed that kids in T1 had the highest level of globulin compared to the other groups. Similarly, garlic supplementation led to enhancing globulin in Asian sea bass (Talpur & Ikhwanuddin, 2012) and African catfish (Thanikachalam *et al.*, 2010). Changes in the

physiological status are often reflected by changes in haematology parameters. Hence, blood indices are important tools which are used to confirm the effects of nutritional and environmental management on animals. Higher RBC, Hb and PCV values were observed in kids fed garlic supplement. Similar to these results, Toghyani *et al.* (2011) reported that RBC, Hb and PCV in broiler chicks increased significantly with garlic supplementation. Similarly, Talpur & Ikhwanuddin (2012) reported that garlic supplementation resulted in increased RBC, WBC, PCV, Hb, phagocytic activity, respiratory burst, lysozyme, anti-protease and bactericidal activities in Asian sea bass. In this study, RBC and Hb were higher in the treated kids compared to the others. In this case, Toghyani *et al.* (2011) suggested that garlic may possess ingredients that stimulate the erythropoietic system to produce more red blood cells. These ingredients would play a role in the stimulation of the immune system and in the function of organs related to blood cell formation (Li *et al.*, 2002). Also, garlic extract is an active oxygen scavenger and therefore it is possible that garlic ingredients challenge with Hb in the RBC for oxygen, resulting in hypoxia which then stimulates Hb synthesis and RBC production. It is also possible that the end-products of garlic metabolism in the body directly stimulate the kidney for the production and secretion of erythropoietin (Toghyani *et al.*, 2011).

In the present study, garlic supplementation also led to enhanced WBC and lymphocyte concentrations but reduced that of neutrophils. In accordance with our results, garlic juice (200 mg kg⁻¹) supplementation in rats significantly enhanced RBC, Hb, PCV, WBC and the lymphocytes compared with control group but in contrary with the present results neutrophils were increased (Iranloye, 2002). Similarly, fermented garlic powder increased WBC, lymphocytes and IgG in broilers (Ao *et al.*, 2011). Moreover, significantly increased WBC and RBC counts following garlic peel feeding have been reported in African catfish (Thanikachalam *et al.*, 2010). In contrary with our results, no positive effect of garlic powder supplementation was observed on lymphocyte, WBC and RBC in finishing pigs (Chen *et al.*, 2008; Yan *et al.*, 2011). Also, our findings was in disagreement with those of Yan *et al.* (2011) who have reported dietary fermented garlic by *Weissella koreensis* powder doesn't affect on lymphocyte, WBC and RBC in growing pigs.

Measurement of immune reactivity is an important tool in determining how animals face environmental demands (Hessing *et al.*, 1995), and is also beneficial as a complement to diagnostic tests based on the immune response. PHA, a lectin from *Phaseolus vulgaris*, led to

agglutination of erythrocytes, and growth, division, and non-specific activation of T-cells. The skin test involves intradermal injecting PHA and measuring the change in skin thickness. The results of the current study indicated that supplementation of garlic led to better response to PHA compared with the control. There are no previous reports about measuring of cellular immune response to PHA in different animals after feeding garlic supplementation. Wang *et al.* (2011) reported that immune response in pigs given 2 g of fermented garlic by *Weissella koreensis* powder per kg body weight after an *Escherichia coli* lipopolysaccharide challenge improved in comparison with control pigs. The main active component of garlic that may have great impact on immunity is allicin. Kyo *et al.* (1998) also suggested that allicin species show immune increasing activities that consist of boosting of lymphocyte synthesis, cytokine release, phagocytosis, and natural killer cell activity. Indeed, results of the present study showed that supplementation with garlic extract increased globulin, WBC, lymphocytes, RBC, Hb and PCV concentrations and immune response to PHA injection, indicating that garlic extract supplementation in milk stimulated the immune system in newborn goat kids.

5 Conclusion

In conclusion, the results of the present study indicate that administration of aqueous garlic extract supplemented in milk (250 mg per kg BW per day) enhanced both growth rate and cell-mediated immune response in newborn goat kids. The 125 mg per kg BW per day treatment had the highest impact on RBC, Hb, PCV, WBC and lymphocytes. Therefore, the administration of garlic extract to newborn goats could be used, also by smallholders, to improve growth and immunological status during the first month of life. However, more investigations are needed to determine the optimal doses.

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