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Abstract: Recognized by the traditional medicine and recent scientific research studies, Lycium barbarum berries (Goji berries) have beneficial effects on human and animal health. The aim of our study was to evaluate the effects of Goji berries on the productive performance of rabbits. One month before insemination, 60 New Zealand White does were randomly assigned to one of the following 3 dietary treatments: commercial standard diet (C); C supplemented with 1% Goji berries (LG); and C supplemented with 3% Goji berries (HG). After weaning up to 91 days of age, 15 randomly selected rabbits/group were fed the same diet as the mothers (C, LG, and HG). Non-pregnant and lactating does of C group showed the highest feed intake (P<0.01), although no significant differences in body weight (BW) were observed between groups. Nutritional treatment did not affect the offspring's feed intake. However, the rabbits fed with Goji supplementation showed not only higher mean BW both during growth (P<0.001) and at slaughter (P<0.01), but also better feed conversion ratio (FCR; P<0.01) than the control group. LG does showed the highest milk production (P<0.001). Moreover, LG group showed lower pre-weaning mortality (P<0.05), higher litter size (P<0.05), higher litter weight (P<0.05) at day 18, and higher litter size at weaning (P=0.05) compared to C group. Although further studies are needed, our study paves the way for the use of Goji berries in the zootechnical field, which could be beneficial not only for animal welfare and livestock productivity but also for new marketing strategies.

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Highlights

Goji berries have been recognized as having beneficial properties in humans

For the first time, the effects of Goji supplementation were evaluated on the rabbit

Goji supplementation improved the pre and post weaning productive performance

The use of Goji berries is proposed for livestock nutrition

Effects of Goji berries supplementation on the productive performance of rabbit

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Abstract

Recognized by the traditional medicine and recent scientific research studies, *Lycium barbarum* berries (Goji berries) have beneficial effects on human and animal health. The aim of our study was to evaluate the effects of Goji berries on the productive performance of rabbits. One month before insemination, 60 New Zealand White does were randomly assigned to one of the following 3 dietary treatments: commercial standard diet (C); C supplemented with 1% Goji berries (LG); and C supplemented with 3% Goji berries (HG). After weaning up to 91 days of age, 15 randomly selected rabbits/group were fed the same diet as the mothers (C, LG, and HG). Non-pregnant and lactating does of C group showed the highest feed intake (P<0.01), although no significant differences in body weight (BW) were observed between groups. Nutritional treatment did not affect the offspring's feed intake. However, the rabbits fed with Goji supplementation showed not only higher mean BW both during growth (P<0.001) and at slaughter (P<0.01), but also better feed conversion ratio (FCR; P<0.01) than the control group. LG does showed the highest milk production (P<0.001). Moreover, LG group showed lower pre-weaning mortality (P<0.05), higher litter size (P<0.05), higher litter weight (P<0.05) at day 18, and higher litter size at weaning (P=0.05) compared to C group. Although further studies are needed, our study paves the way for the use of Goji berries in the zootechnical field, which could be beneficial not only for animal welfare and livestock productivity but also for new marketing strategies.

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1. Introduction

Goji (goji berry or wolfberry) is the fruit of *Lycium barbarum* L., a shrubbery that is widely distributed in the arid and semi-arid regions of Northwestern China, Southeastern Europe, and Mediterranean countries (Jin et al., 2013; Potterat, 2010). For more than 2300 years, the Goji berry has been used as both a traditional medicinal herb and food supplement in East Asia (Cheng et al., 2015; Jin et al., 2013; Potterat, 2010). Because of its potential health benefits, the Goji berry has become increasingly popular in Western countries (Cheng et al., 2015; Jin et al., 2013; Potterat, 2010). In fact, Goji berries seem to have a large variety of beneficial properties: antioxidant and anti-aging (Ceccarini et al., 2016; Li et al., 2013, 2007; Luo et al., 2004; Xia et al., 2014; Ye et al., 2015), antitumoral and immunostimulator (Ceccarini et al., 2016; Chen et al., 2015, 2009; Gan et al., 2003; Mo et al., 2016), gastrointestinal-protective (Kang et al., 2017; Philippe et al., 2012; Zhao et al., 2014), anti-diabetic (Cai et al., 2015; Luo et al., 2004; Todini et al., 2007; Zhao et al., 2016), cardioprotective (Xin et al., 2011b, 2011a), and retinoprotective (Li et al., 2013; Wang et al., 2014). Polysaccharides, which are the major active ingredients of Goji berry, are responsible for the above-mentioned beneficial properties (Chen et al., 2009; Gan et al., 2003; Jin et al., 2013; Jing et al., 2009; Xia et al., 2014; Xin et al., 2011b; Zhao et al., 2014). In addition, Goji berries are a source of carotenoids, vitamins (particularly riboflavin, thiamine, and ascorbic acid), flavonoids, and amino acids with proline as the major constituent (Cheng et al., 2015; Potterat, 2010).

Laboratory animals, mostly mice and rats, have been used as experimental models to study the effects and mechanisms of action of Goji berries (Chen et al., 2015, 2009; Kang et al., 2017; Li et al., 2013, 2007; Wang et al., 2014; Zhao et al., 2014). However, the welfare of other animals, including livestock species, can be improved by the beneficial and nutritional properties of Goji berries. To our knowledge, up to now, Goji berries have not been used as a feed supplement for domestic animals, including the rabbit. Adding Goji berries in animal diet would be in accordance with the European Community guidelines that encourage the use of new biological compounds in order to reduce the use of synthetic drugs such as antibiotics and hormones.

The rabbit is not only a good animal model but also a pet and a livestock species (Brecchia et al., 2014; Collodel et al., 2015; Menchetti et al., 2015a, 2015b). Estimates suggest that 1.2 billion rabbits are globally slaughtered every year to produce meat, and the production is concentrated in China and Europe (FA0STAT 2012). In addition, as rabbits require minimum initial investments and relative ease of management, they are an important source of income in developing countries such as Africa, Central America, and Southeast Asia. Optimizing the nutritional and health aspects of rabbit husbandry could, therefore, increase the productivity of farms in addition to animal welfare (Brecchia et al., 2014; Lebas et al., 1997; Menchetti et al., 2018a). Furthermore, the use of nutraceutical products for rabbit rearing could be a good marketing strategy.

Therefore, the study of Goji berry in rabbit nutrition could offer several strategic advantages: (i) rabbits represent proper experimental models for humans and animals that are useful to understand Goji berries polysaccharides mechanism of action; (ii) Goji berry dietary supplementation may improve rabbit’s productive performance and reduce the incidence of sanitary problems linked to infections and inflammatory status; (iii) Goji berry dietary supplementation may contribute to the production of organic feeds and revive the rabbit meat market.

The aim of this study was to evaluate the effects of a dietary supplementation with two different concentrations of Goji berries, 1% and 3%, on the productive parameters of the rabbit.

2. Materials and methods

The trial was carried out at the experimental farm of the Department of Agricultural, Food, and Environmental Science of the University of Perugia. Rabbits underwent a continuous photoperiod of 16 hours light per day at 40 lux. Room temperature ranged from 18 to 27 °C. Fresh water was always available. The study was conducted in accordance with Legislative Decree No. 146, implementing Directive 98/58/EC of 20 July 1998 concerning the protection of animals kept for farming purposes.

2.1. Rabbit does

Sixty New Zealand White nulliparous does were randomly assigned to three dietary groups (n=20/group): Control (C), Low Goji (LG), and High Goji (HG). Does of C group were fed with a commercial pelleted feed, while does of LG and HG were fed the same feed supplemented with 1% and 3% Goji berries, respectively (Table 1). Goji berries were provided by Impresa Agricola of Gianluca Bazzica, Foligno (Italy). The animals were submitted to artificial insemination (AI) after 30 days of adaptation to the experimental diets. During the period of adaptation, they received 150 g/d of feed. After AI, the rabbits had ad libitum access to feed. Ovulation was induced by injecting 0.8 μg of synthetic GnRH (Receptal, Hoechst-Roussel Vet, Milan, Italy) just before AI (Brecchia et al., 2006). AI was performed with 0.5 ml of diluted fresh semen. Pregnancy was diagnosed by manual palpation 10 days after AI, and non-pregnant does were submitted to successive AI after 21 days in order to achieve 15 pregnant does and 45 kits per group. Lactation was controlled until day 18 post-partum by opening the nest once a day for 5-10 minutes. During the trial, rabbit does' feed intake was recorded daily, while body weight (BW) was measured weekly, between 8:00 and 10:00 A.M.. In addition, milk production was evaluated daily from parturition until day 18 of lactation by weighting the doe immediately before and after suckling (Menchetti et al., 2018b).

2.2. Offspring

After the 18th day of lactation, the nest was open, so the kits had access to the solid feed of mothers. At 35 days of age (weaning), rabbits were separated from their mothers and were moved into individual cages. Then, 3 rabbits/does (45 rabbits/group, n=135) were randomly chosen and fed with the same diet as their mothers (C, LG, and HG) until slaughtering (91 days of age). The slaughter was performed in accordance with the standard procedure practiced in commercial abattoirs.

During the experimental period, the following parameters were evaluated: (i) weight of the litter (n=45) until day 35 and individual BW (n=135) until day 91; (ii) litter size; (iii) pre and post-weaning mortality, (iv) feed conversion ratio (FCR) from 36 to 90 days of age. The pre-weaning mortality rate was calculated as the percentage of weaned kits/litter size following the perinatal period (i.e., the first 48 h after parturition; Menchetti et al., 2018b).

2.3. Statistical analysis

Data were analysed using the Linear Mixed Model, and they were stratified according to the category (rabbit does and offspring) and to the physiological state (pregnant, non-pregnant, and lactating does) of the animals. The models evaluated the effects of the group (3 levels: C, LG, and HG), time (as days, depending on the dependent variable, the physiological state, and category of animals), and group x time interaction. The time was included as a repeated factor using diagonal covariance structure, and it was excluded for non-repeated measures. The dependent variable “Litter weight” was analysed using two different models to evaluate both the total production and the BW of the individual animals: in the first model, no adjustments were used, while in the second model, the “Litter size” was included as a covariate. The Sidak method was used for multiple comparisons. The assumptions were verified by using Kolmogorov-Smirnov test and diagnostic graphics. Results were expressed as estimated marginal means ± standard error (SE) although raw data were presented in figures. Non-parametric statistics was used to analyse FCR (Kruskal-Wallis and Mann-Whitney tests), and the results were expressed as median (Mdn) and interquartile range (IQR). The Chi square test was used to assess mortality. Statistical analyses were performed with SPSS Statistics version 23 (IBM, SPSS Inc., Chicago, IL, USA). $P \leq 0.05$ was defined as significant.

3. Results

3.1. Feed intake of rabbit does and offspring

Feed intake of non-pregnant, pregnant, and lactating does was influenced by group (P<0.01), time (P<0.01), and by their interaction (P<0.05; Fig. 1). Non-pregnant C does (150±1 g/d) showed higher mean feed intake than that recorded for the LG (147±1 g/d; P<0.001) and HG (148±1 g/d; P<0.01) groups. During pregnancy, HG group showed higher feed intake (148±1 g/d) than C (143±1 g/d; P<0.001) and LG (145±1 g/d; P<0.05) groups. Conversely, during lactation, C group showed the highest feed intake (320±3 g/d, 302±3 g/d, and 288±4 g/d in C, LG, and HG, respectively; P<0.01; Fig. 1).

The offspring’s post-weaning feed intake increased from day 35 (89±6 g/d) to 90 (296±8 g/d; P<0.001) days of age and it was not affected by group (181±1 g/d, 179±2 g/d, and 183±2 g/d in C, LG, and HG, respectively; P=0.274).

3.2. Body weight of rabbit does

Body weight during the first month of the dietary treatment was influenced by time (P<0.001), with a progressive increase from the baseline until the AI day (mean difference: 289±88 g; P<0.05). At AI, there were no differences in BW between groups (3872±83 g, 3915±112 g and 4054±96 g in C, LG, and HG respectively; P=0.350).

Similarly, does' BW during pregnancy was only influenced by time (P<0.001), with a significant increase in the last two weeks of pregnancy (P<0.01).

During the lactation period, BW of does (measured before lactation) was not influenced by any factor.

3.3. Pre-weaning performance

Milk production was influenced by dietary group (P<0.001) and time (P<0.001). Milk production increased from the 1st (41±5 g/d) to the 18th day post-partum when it reached the lactation peak (161±6 g/d; P<0.001). LG showed the highest mean milk production, while HG the lowest (113±2 g/d, 129±2 g/d, and 103±2 g/d in C, LG, and HG, respectively; P<0.001; Fig. 2).

Table 2 reports the productive performance of the three groups. The LG group showed lower pre-weaning mortality (P<0.05) and higher litter size at both day 18 (P<0.05) and weaning (P=0.05) compared to C group. When the litter size was not included as covariate in the model, the LG group also showed higher litter weight at day 18 than C group (estimated mean difference: +263±96 g, P<0.05).

3.4. Post-weaning performance

Rabbits' post-weaning BW was influenced by dietary group (P<0.001) and time (P<0.001; Fig. 3). Mean BW increased from 668±18 g at day 42 to 2199±21 g at day 91 (P<0.001). The rabbits fed with the Goji supplementation showed higher mean BW (1240±12 g, 1347±12 g and 1383±12 g for C, LG, and HG, respectively; $P<0.001$) and lower FCR (Mdn = 3.9, IQR = 3.4-5.6, Mdn = 3.3, IQR = 3.0-4.2, and Mdn = 3.5, IQR = 3.0-4.2, for C, LG, and HG, respectively; $P<0.01$) compared to the C group.

Similarly, at slaughter, the C group showed the lowest BW (2072±32 g, 2212±28 g, and 2313±32 g for C, LG, and HG, respectively; $P<0.01$).

4. Discussion

According to our knowledge, this study is the first report on the possible use of Goji berries in rabbit's nutrition. Our work shows encouraging results and suggests attractive applications of Goji berries in livestock nutrition although further studies are needed to assess its practical and economical convenience. Indeed, the present study demonstrates that Goji berry dietary supplementation reduced mortality during the pre-weaning period and improved productive traits in growing rabbits.

The interest of the scientific community and pharmaceutical companies in the beneficial effects of Goji polysaccharides on human and animal health has increased in the last few years (Cheng et al., 2015; Jin et al., 2013; Potterat, 2010). The numerous beneficial properties of the Goji berries can be exploited in farms in order to reduce the use of antibiotics as well as improve both reproductive functions and animal welfare. However, only a few studies concerning the effects of Goji berries on the productive performance of animals have been conducted. In addition, most studies have used mice and rats as laboratory animal models, while there are few studies concerning livestock species.

Goji was assessed for its antioxidant properties to reduce pre-slaughter stress in pigs (Bai et al., 2016). However, only one week of nutritional treatment was provided to the animals in this study, and the authors did not evaluate the effects of Goji berries on pigs' growth performance. In a review, Clément et al. (2012) considered medicinal herbs including Goji berries as potentially useful feed supplements to improve male reproductive performance of livestock. However, they highlighted the lack of data on livestock and referred to bibliographic sources regarding other mammals, especially rodents. Some authors (Luo et al., 2004; Zhao et al., 2016) investigated the effect of Goji berries on diabetic rabbits. They administered Goji fruit and/or polysaccharide fractions to rabbits treated with alloxan. In accordance with our study, these authors did not find side effects associated with the administration of the Goji berry to rabbits. Conversely, they showed that Goji berry has both hypoglycaemic and hypolipidemic effects, improves the renal function, and alleviates the inflammatory reaction in the kidneys of diabetic rabbits (Luo et al., 2004; Zhao et al., 2016).

Our research focused on the effects of the administration of two different concentrations of Goji berries, 1% and 3%, as a supplement to a conventional diet, on the productive performance of rabbits. Results showed that Goji supplementation reduced feed intake in non-pregnant does without affecting the body weight which suggests an improved feed conversion rate for maintenance. Actually, the significant differences in feed intake between groups were of dubious biological relevance in non-pregnant does although they increased during lactation. More importantly, supplemented rabbits reached higher body weights and had better feed conversion ratio after weaning compared to the control group. Although the mean differences in feed intake between groups were a few grams per day, this result could be of great interest to the market since animal feeding is the most expensive factor in rabbit breeding. For this reason, these data have to be confirmed by other studies aimed at assessing the cost-effectiveness of Goji berries in animal nutrition.

To date, the administration of Goji berries to enhance growth has been used only in fishes (Mo et al., 2016). Goji berries increased the body weight of diabetic mice (Jing et al., 2009) and reduced body weight loss in induced colitis models regardless of feed intake (Kang et al., 2017; Philippe et al., 2012). Conversely, Cheng and Kong (2011) found no effects of *Lycium barbarum* polysaccharide administration on body weight of rats with alcohol-induced liver damage. Bai et al. (2016) have obtained a similar result in pigs fed diets with 1% Goji berry supplementation. On the other hand, several studies (Kang et al., 2010; Kim et al., 2017; Xiao et al., 2014) showed that Goji berries reduced body weight in obese subjects. Further studies are needed to understand how they interact with the digestive system of mammals. However, it may be speculated that the enhanced feed conversion rate may be due to changes in the microbial communities and digestive enzymes, which improve antioxidant and anti-inflammatory activities, metabolic balance, and intestinal function (Amagase and Nance, 2008; Kang et al., 2017; Mo et al., 2016; Philippe et al., 2012).

The does supplemented with 1% Goji showed the highest milk production. This may have positively influenced the pre-weaning productive performance. In fact, the same group showed lower pre-weaning mortality and higher litter size compared to the control group. Although there are no studies evaluating the effects of Goji in milk production and composition, changes in the immunomodulatory properties of milk can be speculated.

It should be noted that the best reproductive performances were achieved with the low-dose supplementation. The lowest milk production of does supplemented with 3% Goji and their subsequent mediocre reproductive performances could be attributed to the low feed intake during lactation. This finding suggests that the bioactive compounds contained in the Goji berries could have an anti-nutritional activity at high concentrations and in the long-term. It must be stressed that an excessive intake of polyphenols was proved to have pro-oxidant effects in both broilers (Branciari et al., 2016) and rabbits (Dal Bosco et al., 2012). Further investigations are required to clarify the mechanism of action of these compounds and their interactions with the oxidative metabolism of the animal.

Finally, supplementation with Goji berries improved the general well-being of the animals as evidenced by their very thick and shiny coat, increased motor activity, and a lower incidence of leg injuries and abscesses compared to control group (data not shown). Two meta-analyses of human clinical studies (Amagase and Hsu, 2009; Paul Hsu et al., 2012) showed that Goji berry consumption enhances the general well-being, neurologic/psychologic traits, and gastrointestinal functions.

5. Conclusion

In conclusion, 1% Goji supplementation not only increased milk production and reduced pre-weaning mortality but also improved growth rate and rabbit welfare. Further studies regarding the effective increase of farm productivity, cost analysis, the presence of anti-nutritional compounds, and the meat quality are required. However, our findings on rabbits are encouraging and suggest potential applications of Goji berries in other livestock species nutrition. In fact, the use of these nutraceutical substances could be advantageous both for animal welfare and livestock productivity. Moreover, the use of natural products for the meat production could represent a marketing strategy to give new input to the breeding of the rabbit.

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Conflict of interest

The authors declare that they have no competing interests.

References

Amagase, H., Hsu, C.-H.P., 2009. Meta-analysis of the General Effects of a Standardized Lycium barbarum Fruit Juice Shown in Randomized, Double-blind, Placebo-controlled Human Clinical Studies. FASEB J. 23, 716.

Amagase, H., Nance, D.M., 2008. A randomized, double-blind, placebo-controlled, clinical study of the general effects of a standardized Lycium barbarum (Goji) Juice, GoChi. J. Altern. Complement. Med. 14, 403–412. doi:10.1089/acm.2008.0004

Bai, X., Yan, X., Xie, L., Hu, X., Lin, X., Wu, C., Zhou, N., Wang, A., See, M.T., 2016. Effects of pre-slaughter stressor and feeding preventative Chinese medicinal herbs on glycolysis and oxidative stability in pigs. Anim. Sci. J. 87, 1028–1033. doi:10.1111/asj.12537

Branciari, R., Ranucci, D., Ortenzi, R., Roila, R., Trabalza-Marinucci, M., Servili, M., Papa, P., Galarini, R., Valiani, A., 2016. Dietary administration of olive millwastewater extract reduces campylobacter spp. prevalence in broiler chickens. Sustain. 8, 3–9. doi:10.3390/su8090837

Brecchia, G., Menchetti, L., Cardinali, R., Castellini, C., Polisca, a., Zerani, M., Maranesi, M., Boiti, C., 2014. Effects of a bacterial lipopolysaccharide on the reproductive functions of rabbit does. Anim. Reprod. Sci. 147, 128–134. doi:10.1016/j.anireprosci.2014.04.009

Cai, H., Liu, F., Zuo, P., Huang, G., Song, Z., Wang, T., Lu, H., Guo, F., Han, C., Sun, G., 2015. Practical Application of Antidiabetic Efficacy of Lycium barbarum Polysaccharide in Patients with Type 2 Diabetes. Med Chem 11, 383–390.

Ceccarini, M.R., Vannini, S., Cataldi, S., Moretti, M., Villarini, M., Fioretti, B., Albi, E., Beccari, T., Codini, M., 2016. Effect of Lycium barbarum berries cultivated in Umbria (Italy) on human hepatocellular carcinoma cells. J. Biotechnol. 231, S26–S27.

doi:10.1016/j.jbiotec.2016.05.114

Chen, S., Liang, L., Wang, Y., Diao, J., Zhao, C., Chen, G., He, Y., Luo, C., Wu, X., Zhang, Y., 2015. Synergistic immunotherapeutic effects of Lycium barbarum polysaccharide and interferon-α2b on the murine Renca renal cell carcinoma cell line in vitro and in vivo. Mol. Med. Rep. 12, 6727–6737. doi:10.3892/mmr.2015.4230

Chen, Z., Lu, J., Srinivasan, N., Tan, B.K.H., Chan, S.H., 2009. Polysaccharide-protein complex from Lycium barbarum L. is a novel stimulus of dendritic cell immunogenicity. J. Immunol. 182, 3503–3509. doi:10.4049/jimmunol.0802567

Cheng, D., Kong, H., 2011. The effect of lycium barbarum polysaccharide on alcohol-induced oxidative stress in rats. Molecules 16, 2542–2550. doi:10.3390/molecules16032542

Cheng, J., Zhou, Z.-W., Sheng, H.-P., He, L.-J., Fan, X.-W., He, Z.-X., Sun, T., Zhang, X., Zhao, R.J., Gu, L., Cao, C., Zhou, S.-F., 2015. An evidence-based update on the pharmacological activities and possible molecular targets of Lycium barbarum polysaccharides. Drug Des. Devel. Ther. 9, 33–78. doi:10.2147/DDDT.S72892

Clément, C., Witschi, U., Kreuzer, M., 2012. The potential influence of plant-based feed supplements on sperm quantity and quality in livestock: A review. Anim. Reprod. Sci. 132, 1–10. doi:10.1016/j.anireprosci.2012.04.002

Collodel, G., Moretti, E., Brecchia, G., Kuželová, L., Arruda, J., Mourvaki, E., Castellini, C., 2015. Cytokines release and oxidative status in semen samples from rabbits treated with bacterial lipopolysaccharide. Theriogenology 83, 1233–1240. doi:10.1016/j.theriogenology.2015.01.008

Dal Bosco, A., Mourvaki, E., Cardinali, R., Servili, M., Sebastiani, B., Ruggeri, S., Mattioli, S., Taticchi, A., Esposto, S., Castellini, C., 2012. Effect of dietary supplementation with olive pomaces on the performance and meat quality of growing rabbits. Meat Sci. 92, 783–788.

doi:10.1016/j.meatsci.2012.07.001

Gan, L., Zhang, S.-H., Liu, Q., Xu, H.-B., 2003. A polysaccharide-protein complex from Lycium barbarum upregulates cytokine expression in human peripheral blood mononuclear cells. Eur. J. Pharmacol. 471, 217–222. doi:10.1016/S0014-2999(03)01827-2

Jin, M., Huang, Q., Zhao, K., Shang, P., 2013. Biological activities and potential health benefit effects of polysaccharides isolated from Lycium barbarum L. Int. J. Biol. Macromol. 54, 16–23. doi:10.1016/j.ijbiomac.2012.11.023

Jing, L., Cui, G., Feng, Q., Xiao, Y., 2009. Evaluation of hypoglycemic activity of the polysaccharides extracted from Lycium barbarum. African J. Tradit. Complement. Altern. Med. 6, 579–584.

Kang, M.-H., Park, W.-J., Choi, M.-K., 2010. Anti-obesity and hypolipidemic effects of Lycium chinense leaf powder in obese rats. J. Med. Food 13, 801–7. doi:10.1089/jmf.2010.1032

Kang, Y., Xue, Y., Du, M., Zhu, M.-J., 2017. Preventive effects of Goji berry on dextran-sulfate-sodium-induced colitis in mice. J. Nutr. Biochem. 40, 70–76. doi:10.1016/j.jnutbio.2016.10.009

Kim, M.H., Kim, E.-J., Choi, Y.Y., Hong, J., Yang, W.M., 2017. Lycium chinense Improves Post-Menopausal Obesity Via Regulation of PPAR-γ and Estrogen Receptor-α/β Expressions. Am. J. Chin. Med. 1–14. doi:10.1142/S0192415X17500173

Lebas, F., Coudert, P., De Rochambeau, H., Thebault, R.G., Rouvier, R., De Rochambeau, H., 1997. The rabbit: husbandry, health and production., FAO Animal. ed, FAO- Food and Agriculture Organization of the United Nations.

Li, H., Liang, Y., Chiu, K., Yuan, Q., Lin, B., Chang, R.C.C., So, K.F., 2013. Lycium Barbarum (Wolfberry) Reduces Secondary Degeneration and Oxidative Stress, and Inhibits JNK Pathway in Retina after Partial Optic Nerve Transection. PLoS One 8.

doi:10.1371/journal.pone.0068881
Li, X.M., Ma, Y.L., Liu, X.J., 2007. Effect of the Lycium barbarum polysaccharides on age-related oxidative stress in aged mice. J. Ethnopharmacol. 111, 504–511. doi:10.1016/j.jep.2006.12.024
Luo, Q., Cai, Y., Yan, J., Sun, M., Corke, H., 2004. Hypoglycemic and hypolipidemic effects and antioxidant activity of fruit extracts from Lycium barbarum. Life Sci. 76, 137–149. doi:10.1016/j.lfs.2004.04.056
Maertens, L., Moermans, R., De Groote, G., 1988. Prediction of the apparent digestible energy content of commercial pelleted feeds for rabbits. J. Appl. Rabbit Res 11, 60–67.
Menchetti, L., Barbato, O., Filipescu, I.E., Traina, G., Leonardi, L., Polisca, A., Troisi, A., Guelfi, G., Piro, F., Brecchia, G., 2018a. Effects of local lipopolysaccharide administration on the expression of Toll-like receptor 4 and pro-inflammatory cytokines in uterus and oviduct of rabbit does. Theriogenology 107, 162–174. doi:10.1016/j.theriogenology.2017.10.046
Menchetti, L., Brecchia, G., Canali, C., Cardinali, R., Polisca, A., Zerani, M., Boiti, C., 2015a. Food restriction during pregnancy in rabbits: Effects on hormones and metabolites involved in energy homeostasis and metabolic programming. Res. Vet. Sci. 98, 7–12. doi:10.1016/j.rvsc.2014.11.017
Menchetti, L., Brecchia, G., Cardinali, R., Polisca, A., Boiti, C., 2015b. Food restriction during pregnancy: effects on body condition and productive performance of primiparous rabbit does. World Rabbit Sci. 23, 1–8. doi:10.4995/wrs.2015.1703
Menchetti, L., Canali, C., Castellini, C., Boiti, C., Brecchia, G., 2018b. The different effects of linseed and fish oil supplemented diets on insulin sensitivity of rabbit does during pregnancy. Res. Vet. Sci. 118, 126–133. doi:10.1016/j.rvsc.2018.01.024

Mo, W.Y., Lun, C.H.I., Choi, W.M., Man, Y.B., Wong, M.H., 2016. Enhancing growth and non-specific immunity of grass carp and Nile tilapia by incorporating Chinese herbs (Astragalus membranaceus and Lycium barbarum) into food waste based pellets. Environ. Pollut. 219, 475–482. doi:10.1016/j.envpol.2016.05.055

Paul Hsu, C.-H., Nance, D.M., Amagase, H., 2012. A meta-analysis of clinical improvements of general well-being by a standardized Lycium barbarum. J. Med. Food 15, 1006–14. doi:10.1089/jmf.2012.0013

Philippe, D., Brahmbhatt, V., Foata, F., Saudan, Y., Serrant, P., Blum, S., Benyacoub, J., Vidal, K., 2012. Anti-inflammatory effects of Lacto-Wolfberry in a mouse model of experimental colitis. World J. Gastroenterol. 18, 5351–5359. doi:10.3748/wjg.v18.i38.5351

Potterat, O., 2010. Goji (*Lycium barbarum* and *L. chinense*): Phytochemistry, Pharmacology and Safety in the Perspective of Traditional Uses and Recent Popularity. Planta Med. 76, 7–19. doi:10.1055/s-0029-1186218

Todini, L., Trabalza-Marinucci, M., Malfatti, A., Barbato, O., Stradaioli, G., Cavallucci, C., Debenedetti, A., 2007. Pre- and post-feeding plasma gastrin-17 and insulin concentrations and feed intake in female goats during different physiological stages. Small Rumin. Res. 71, 38–47. doi:10.1016/j.smallrumres.2006.04.007

Wang, K., Xiao, J., Peng, B., Xing, F., So, K.-F., Tipoe, G.L., Lin, B., 2014. Retinal structure and function preservation by polysaccharides of wolfberry in a mouse model of retinal degeneration. Sci. Rep. 4, 7601. doi:10.1038/srep07601

Xia, G., Xin, N., Liu, W., Yao, H., Hou, Y., Qi, J., 2014. Inhibitory effect of Lycium barbarum polysaccharides on cell apoptosis and senescence is potentially mediated by the p53 signaling pathway. Mol. Med. Rep. 9, 1237–1241. doi:10.3892/mmr.2014.1964

Xiao, J., Xing, F., Huo, J., Fung, M.L., Liong, E.C., Ching, Y.P., Xu, A., Chang, R.C.C., So, K.F., Tipoe, G.L., 2014. Lycium barbarum polysaccharides therapeutically improve hepatic functions in non-alcoholic steatohepatitis rats and cellular steatosis model. Sci. Rep. 4, 1–12. doi:10.1038/srep05587

Xin, Y., Wan, L.L., Peng, J.L., Guo, C., 2011a. Alleviation of the acute doxorubicin-induced cardiotoxicity by Lycium barbarum polysaccharides through the suppression of oxidative stress. Food Chem. Toxicol. 49, 259–264. doi:10.1016/j.fct.2010.10.028

Xin, Y., Zhang, S., Gu, L., Liu, S., Gao, H., You, Z., Zhou, G., Wen, L., Yu, J., Xuan, Y., 2011b. Electrocardiographic and Biochemical Evidence for the Cardioprotective Effect of Antioxidants in Acute Doxorubicin-Induced Cardiotoxicity in the Beagle Dogs. Biol. Pharm. Bull. 34, 1523–1526. doi:10.1248/bpb.34.1523

Ye, M., Moon, J., Yang, J., Lim, H.H., Hong, S. Bin, Shim, I., Bae, H., 2015. The standardized Lycium chinense fruit extract protects against Alzheimer's disease in 3xTg-AD mice. J. Ethnopharmacol. 172, 85–90. doi:10.1016/j.jep.2015.06.026

Zhao, L., Wu, H., Zhao, A., Lu, H., Sun, W., Ma, C., Yang, Y., Xin, X., Zou, H., Qiu, M., Jia, W., 2014. The in vivo and in vitro study of polysaccharides from a two-herb formula on ulcerative colitis and potential mechanism of action. J. Ethnopharmacol. 153, 151–159. doi:10.1016/j.jep.2014.02.008

Zhao, Q., Li, J., Yan, J., Liu, S., Guo, Y., Chen, D., Luo, Q., 2016. Lycium barbarum polysaccharides ameliorates renal injury and inflammatory reaction in alloxan-induced diabetic nephropathy rabbits. Life Sci. 157, 82–90. doi:10.1016/j.lfs.2016.05.045

Captions for figures

Figure 1. Feed intake in non- pregnant, pregnant, and lactating rabbit does of control group (C) and supplemented with 1% (LG) and 3% (HG) of Goji. Values are means±SE (raw data). Bars not sharing any superscript within each Physiological status are significantly different at $P<0.05$ (Sidak correction).

Figure 2. Milk production from day 1 to 18 post-partum of control group (C) and does supplemented with 1% (LG) and 3% (HG) of Goji. Values are means±SE (raw data).

Figure 3. Body weight (BW) of rabbits of control group (C) and supplemented with 1% (LG) and 3% (HG) of Goji during post-weaning period (days 42-91 of age). Values are means±SE (raw data).

Table 1. Formulation and chemical composition (as fed) of control (C) and experimental diets supplemented with Goji berries.

	Unit	Diet		
		C	1% Goji	3% Goji
Ingredients				
Wheat bran	%	30.0	29.5	29.0
Dehydrated alfalfa meal	%	42.0	41.5	41.0
Barley	%	9.5	9.5	9.0
Sunflower meal	%	4.5	4.5	4.2
Rice bran	%	4.0	4.0	3.9
Soybean meal	%	4.0	4.0	3.9
Calcium carbonate	%	2.2	2.2	2.2
Cane molasses	%	2.0	2.0	2.0
Vitamin-mineral premix*	%	0.4	0.4	0.4
Soybean oil	%	0.4	0.4	0.4
Salt	%	0.3	0.3	0.3
Goji berries	%	-	1.0	3.0
Analytical data				
Crude Protein	%	15.74	15.64	15.66
Ether extract	%	2.25	2.23	2.47
Ash	%	9.28	9.36	9.25
Starch	%	16.86	17.07	16.99
NDF	%	38.05	38.55	37.49
ADF	%	19.54	19.60	19.01
ADL	%	4.01	4.31	3.98
Digestible Energy**	Kcal/kg	2464	2463	2459

* Per kg diet: vitamin A 11,000 IU; vitamin D3 2000 IU; vitamin B1 2.5 mg; vitamin B2 4 mg; vitamin B6 1.25 mg; vitamin B12 0.01 mg; alpha-tocopherol acetate 50 mg; biotine 0.06 mg; vitamin K 2.5 mg; niacin 15 mg; folic acid 0.30 mg; D-pantothenic acid 10 mg; choline 600 mg; Mn 60 mg; Fe 50 mg; Zn 15 mg; I 0.5 mg; Co 0.5 mg.
** Estimated by Maertens et al. (1988)

Table 2. Pre-weaning performance of control group (C) and does supplemented with 1% (LG) and 3% (HG) of Goji.

Parameter		Time	Group			P value
			C	LG	HG	
Mortality (%)		**Perinatal**	11.0	2.9	8.0	0.078
		Pre-weaning	30.9_{a}	14.7_{b}	$29.0_{a,b}$	**0.019**
Litter size (n°, mean±SE)		**1 day**	6±1	7±1	7±1	0.441
		18 days	5_{a} ±1	7_{b} ±1	$6_{a,b}$±1	**0.026**
		35 day	4_{a}±1	6_{b}±1	$4_{a,b}$±1	**0.052**
Weight (gr, mean±SE)	**of litter[2]**	**1 day**	345±32	398±32	346±37	0.428
		18 days	1278_{b} ±72	1541_{a} ±61	$1319_{a,b}$ ±61	**0.019**
	of litter[2,3]	**1 day**	367±14	375±14	247±16	0.426
		18 days	1394±62	1443±52	1335±46	0.307
	of rabbit	**35 days**	729±116	853±124	886±216	0.704

Values followed by the same letter in each row do not differ significantly (P<0.05). Values in bold show statistically significant results at P≤ 0.05.

[2] evaluated before the lactation. [3] estimated means including in the model the litter size as covariate

*Conflict of Interest Statement

Conflict of Interest declaration

I wish to draw the attention of the Editor to the following facts which may be considered as potential conflicts of interest and to significant financial contributions to this work.

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I confirm that the manuscript has been read and approved by all named authors and that there are no other persons who satisfied the criteria for authorship but are not listed. I further confirm that the order of authors listed in the manuscript has been approved by all of us. I confirm that we have given due consideration to the protection of intellectual property associated with this work and that there are no impediments to publication, including the timing of publication, with respect to intellectual property. In so doing I confirm that we have followed the regulations of our institutions concerning intellectual property.

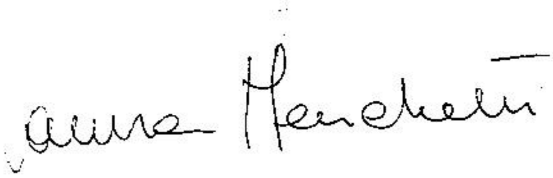

Figure 1

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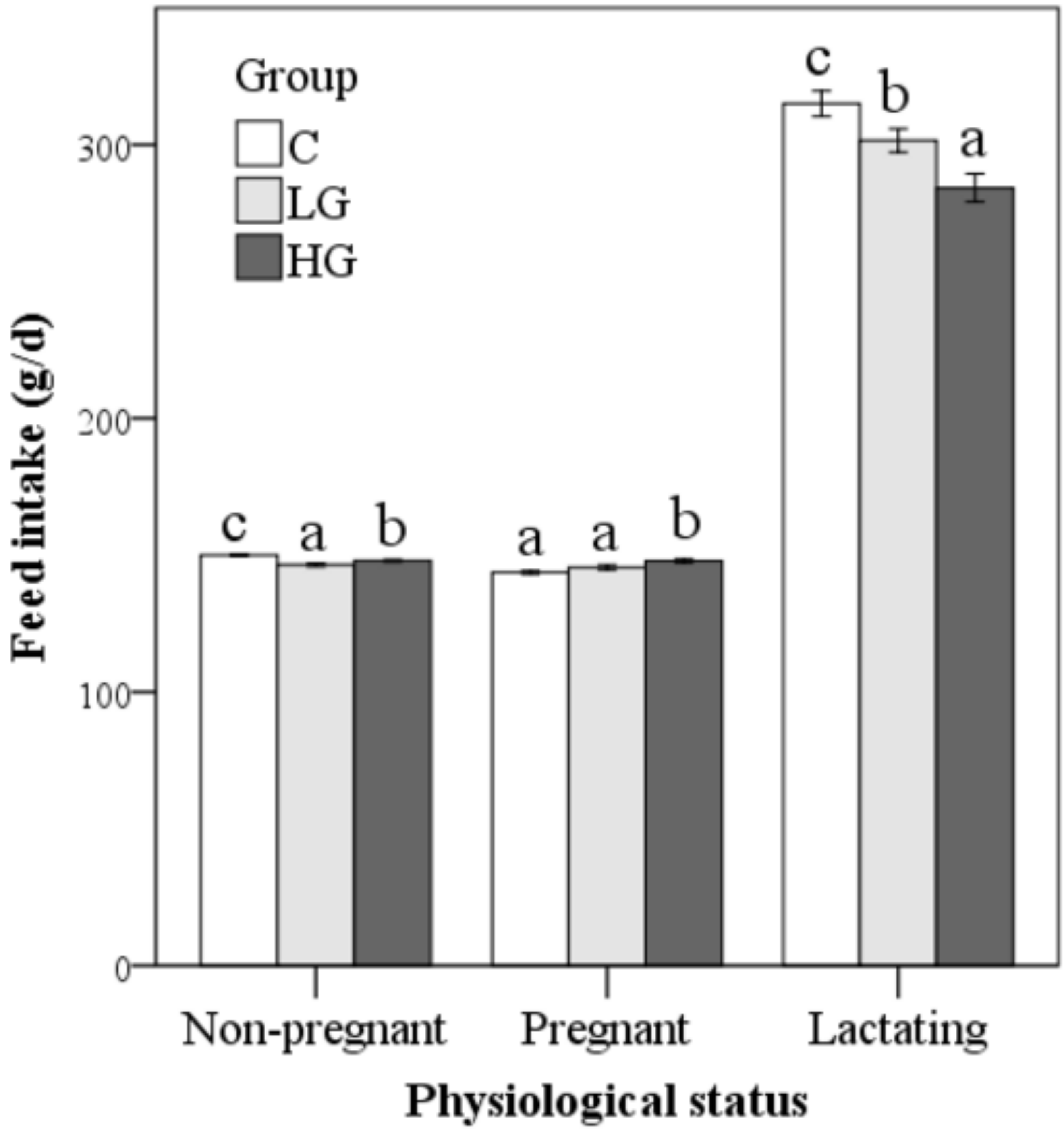

Figure 2

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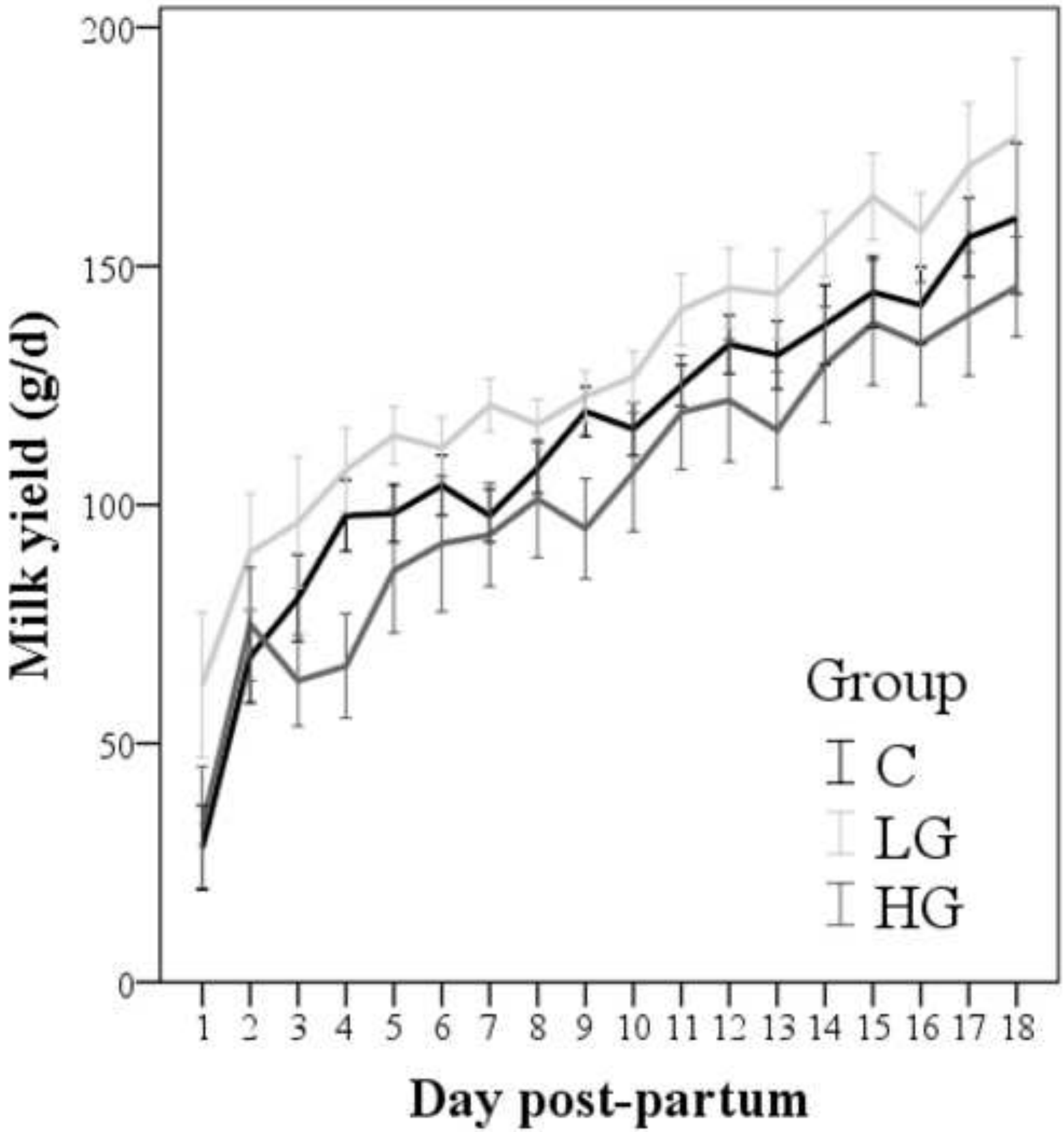

Figure 3
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