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EFFECT OF AQUEOUS EXTRACTS OF GALEGA OFFICINALIS AND ASPARAGUS RACEMOSUS SUPPLEMENTATION ON DEVELOPMENT OF MAMMARY GLAND, MILK YIELD AND ITS IMPACT ON THE PRODUCTIVITY OF RABBIT DOES

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ABSTRACT: The present study was carried out to investigate the effect of aqueous extracts of Galega officinalis and Asparagus racemosus on mammary gland development, milk yield and its impact on the productivity of rabbit does. A total number of sixty female V-Line breed rabbits initially aged 10 weeks old, and extended throughout three sequence parities were used in this study. The animals were randomly distributed into five experimental groups (12 does each) .The 1st group was served as control without any supplementations in drinking water, the 2nd and 3rd groups were supplemented with aqueous extracts from aerial parts of Galega officinalis (G. officianalis) of 150 and 300 mg crude extract /kg body weight (BW) respectively. Whereas, the 4th and 5th groups were supplemented with aqueous extract of Asparagus racemosus (A. racemosus) roots of 100 and 200 mg /kg BW respectively of rabbit does. Addition of A. racemosus and G. officinalis led to a significant increase in values of ovarian hormones and surface area of the mammary alveoli compared to control group. Supplementing the rabbit does with both concentrations of G. officinalis in drinking water during rearing period and continuously for full duration of gestation period and first the 21 days of lactation significantly accelerated pubertal mammary gland development, increase milk yield and enhancing productivity of rabbit does compared to control group throughout three sequence parities. The same trend of significant increase was observed with both concentrations of supplementation with A. racemosus during rearing and lactation only. Whereas, supplementing with high level of A. racemosus (200mg/kg BW) during pregnant period significantly increased stillborn at birth .Also, the rabbit does which treated with A. racemosus for throughout the gestation period exhibited higher fetuses resorption rate at slaughter and aberrant embryo spacing within the uterine horns, and therefore smaller litter size of pups in the post-natal studies, with delayed development and increased mortality. In conclusion**,** water supplementations with aqueous extract from both concentrations of G. officinalis in the drinking water for rabbit does could be recommended to improve pubertal mammary gland development, increase milk yield and enhance productivity of rabbit does. In addition, supplementation with A. racemosus should be avoided in pregnant rabbit does.

Keywords: Rabbits, Galega officinalis, Asparagus racemosus, mammary gland, milk yield

INTRODUCTION

Offspring growth rate during lactation and the mass of offspring at weaning have a significant impact on their subsequent survival, growth and reproductive success in mammals (Hall et al., 2001). The most significant determinant of offspring growth during the lactation period is the ability of females to transfer milk energy to their neonates. Females may vary in their ability to deliver milk energy to their offspring independent of variation in factors such as resource availability and levels of body energy stores or body size (Lang et al., 2009) as a consequence of differences in the capacity of their mammary glands for milk production (Knight, 2000). The real yield of milk is a function of the amount produced by each secretory cell and the number of secretory cells. Hence those factors that determine the epithelial cell population are crucial to the control of milk production (Knight and Peaker, 1982).The mammary gland of primiparous females may have both a lower secretory and storage capacity compared to those of multiparous females (Lang et al., 2012). During early pregnancy, estrogen and progesterone are the key components in the breast tissue development for lactation; estrogen stimulates milk duct development and progesterone forms lobules that are responsible for milk production (Kass et al., 2004).

Many herbal plants contain large number of chemical active principles (phytoestrogens), having galactogenic properties. Among these Goat's Rue (Galega officianalis) and Shatavari (Asparagus racemosus) .Asparagus racemosus was found to demonstrate estrogenic effects in genital organs and in mammary glands in rats with hyperplasia in alveolar tissues and acini accompanied and with increased milk production (Sahay and Pandey, 2003; Sharma and Bhatnagar, 2011). A Same significant trend of increase in milk yield was observed in pigs and goats after feeding with lactare (commercial herbal galactogogues with A. racemosus in its formulation) which also increased growth of the mammary glands, alveolar tissues, and acini (Narendranath et al., 1986).

Galega officianalis may have a positive effect on insulin sensitivity, and is thought to be the forerunner of the popular diabetes drug metformin (Bailey and Day, 2004). Insulin has a direct action on the mammary gland during breast development and is vital to the production and secretion of colostrum (lactogenesis I), in lactogenesis II (when lots of milk arrives after the placenta is born, usually around day 2 or 3 postpartum), and continued lactation (Berlato and Doppler, 2009). Also, literature indicated that 2 phytoestrogens (sativan and medicarpin) isolated from leaves of G. officinalis was cytotoxic against human breast cancer (Le Bail et al., 2000). Gonzalez-Andres et al. (2004) reported that of phytoestrogens at low doses or foods containing G. officinalis could promote activation of some estrogen receptors and increase milk production in sheep. Kahkeshani et al. (2015) indicated that, the positive impact of G. officinalis on milk quantity and quality of cattle can be refer to the active phytochemicals like phenols, flavonoids, saponins, terpenes and sterols.

The previous part of the series researches done by Hamed (2016) proved that phenols and flavonoids presented in water crude extract of aerial parts G. officinalis and A. racemosus roots by phytochemical screening .She supplementing the rabbit does with those extracts in drinking water and reported an enhancing growth performance, antioxidant status and liver function.

The aim of this work was to investigate the effects of whether supplementation aqueous extracts of G. officinalis and/ or A. racemosus in drinking water during rearing period and extended throughout three sequence parities have useful effects on reproductive organs

development of females and their impacts on milk production and rabbit does productivity.

MATERIALS AND METHODS

Preparation and Administration of Aqueous Extracts:

Dried aerial parts of Goat's Rue (Galega officianalis) and root of Shatavari (Asparagus racemosus) were purchased from the local market of Medicinal Plants and Herbs, Cairo, Egypt. The plant was air-dried and grounded to a fine powder by electric dry mill. Aqueous extractions of these herbs were prepared and administered for experimental rabbits by using the same technique mentioned in our previous paper, Hamed (2016).

Animals and Experimental Design:

The experimental work of the present study was carried out at El-Sabahia Poultry Research Station (Alexandria), Animal Production Research Institute during the period from (August to April 2012 - 2013). A total number of sixty female V-Line breed rabbits aged 10 weeks old, were used in this study. Supplementing the rabbit does with G. officianalis and A. racemosus in drinking water was performed during the rearing period (from 10 weeks of age to first insemination) and extended throughout three sequence parities. During the period of reproduction, the experimental supplementations were continuously done during pregnancy up to first 21 day of lactation for the first parity and were added only from birth up to first 21 days of lactation for the second and third parities. The animals were randomly distributed into five experimental groups (12 does each) .The 1st rabbits group was served as a control without any supplementations in drinking water, the 2nd and 3rd groups were supplemented with aqueous extracts of G. officianalis 150 mg and 300 mg (crude water extract) / kg B.W in drinking water for rabbit does, respectively. Whereas, the 4th and 5th groups were supplemented with aqueous extracts of A. racemosus root at 100 mg and 200 mg (crude water extract) /kg B.W in drinking water for rabbit does, respectively.

Dosing solutions were prepared based on average body weight and average daily water consumption. On the assumption that, water consumption per day on average of 300 to 350 mL and females weigh an average of 2.5 to 3.5 kg. Also, doses of aqueous extracts from G. officianalis and A. racemosus were fixed on the basis doses used in earlier studies by different research workers (Luka and Omoniwa, 2012 and Ravishankar et al., 2012).

The rabbits were individually housed in galvanized Italian wire cages (30 × 25 × 40 cm) provided with feeders and automatic stainless steel nipple drinkers. The pelleted diet and fresh water was offered ad libitum. The pellets were 0.62 cm in length and 0.45 cm in diameter. The rabbits were kept under similar managerial and hygienic conditions. The basal diet was composed of 10% maize, 13% barley, 3% molasses, 39.5% clover hay, 15% wheat bran, 17.5% soybean meal, 0.8% dicalcium phosphate, 0.5% limestone, 0.3% sodium chloride, 0.3% vitamin and mineral mixture and 0.1% methionine. The chemical compositions of the basal diet was analysed according to the AOAC (2007).The analysis showed that 90.32% dry matter, 80.8% organic matter, 17.24% crude protein, 13.46% crude fibre, 2.8% ether extract, 9.52% ash and 56.98% nitrogen-free extract. The calculated digestible energy value was 2464 kcal/kg diet. The diet was formulated to meet the nutrient requirements of rabbit does according to NRC (1977).

Each rabbit doe was transferred to the buck's cage for mating and returned to its cage after copulation. Each doe was subjected to two services by the same buck. Each buck mated 1-5 does and each doe palpated 10 days thereafter to detect pregnancy. Those which failed to conceive were returned to the same buck at the same day of test. Does were presented to males

not earlier than 10 days after parturition and then daily until effective mating. Young rabbits were weaned at 28 days. Parameters of females and their offspring were studied between birth days until weaning age (28 days) across three consecutive reproductive cycles. Litter traits such as litter size and weight from birth to weaning and postnatal pup survival were calculated.

Five rabbit does from each group were randomly taken at the first pregnancy (premiparous) at the 2nd half of pregnancy, fasted for 6 hours, individually weighed and slaughtered . Blood samples were collected from each animal; blood was centrifuged at 3000 rpm for 10 min. The serum was frozen stored at .-20º C until progesterone and estradiol were measured. The entire reproductive tract was removed after slaughter. The weight of each reproductive tract was taken after which each ovary was carefully removed from its ovarian bursa at the end of its infundibulum. Pituitary gland, brain and ovaries were separated and weighed to the nearest 0.001 g. The mammary glands were dissected free of the skin and of extraneous muscular, connective and lymphoid tissues. Mammary glands were immediately weighed. Samples of the right side were taken on the posterior portion of the gland. Mammary glands were stored in 10% formalin. The carcass parameters were: dressing percentage (weight of hot eviscerated carcass including abdominal fat divided by the live body weight); organs weight as a percentage of live body weight (liver, kidney, spleen, and reproductive organs of females).

Histometric Measurements of the mammary gland

The mammary gland was removed from rabbits under ether anesthesia preserved in 10% formalin, fixed in Bouin's solution, dehydrated in ascending grades of alcohol, cleared in xylol and cut at 5 micron. These techniques were done according to Bancroft et al. (1994). Randomly selected five fields under a light microscope of each of the five slides representing each treatment from mammary gland were projected at a magnification x4, 10, 40 and then the outline of the different structures in the field was measured using micrometer.

The distance between lobes, distance between lobules, thickness of myoepithelial layer of the mammary alveolus, surface area and diameter for each lobe and each lobules and surface area and the number of each alveolus by mammary alveoli were measured.

Milk yield was recorded daily for the entire lactation period as average weights of both doe and bunnies before and after suckling. The bunnies were separated from their mothers at 15.00 pm, thereafter the bunnies were allowed to suckle at 8.00 am in the next day. The average of the differences between weight of each doe and their bunnies before and after suckling were calculated. Milk yield and body weight of dams and weight gain of pups were determined each day.

Data Analysis:

Data were analyzed as a randomized design using the General Linear Model procedure of SAS (2004). The statistical model was:

$Y_{ij}=U+T_i+e_{ij}$

Y_{ij} = An observation,

U=overall mean

T_i =Effect 0f treatment

e_{ij}=Random error

The above statistical model was more fitted for all studied traits included first parity. Second and third parities were analyzed using the following model:

$Y_{ijk}=\mu+T_i+P_j+e_{ijk}$

Y_{ijk} = an observation,

μ = overall mean

T_i = fixed effect of treatment

P_j= fixed effect of parity

e_{ijk}= residual error

The Duncan's multiple range tests (Duncan, 1955) were used to elucidate differing means. $P\leq0.05$ were accepted as significant.

RESULTS AND DISCUSSION

The effect of aqueous extract of G. officinalis and A. recemosus supplementation on estrogen and progesterone hormones of rabbit does are demonstrated in Figure 1. The highest significant value of estrogen was recorded for the rabbit group on G. officinalis at high level (300 mg/kg B.W) compared with the rest groups. Moreover, different concentrations of supplementations showed significant increase value in estrogen compared to control .The same trend of significant increase of estrogen was observed for progesterone hormone.

Increasing estrogen and progesterone hormones due to supplemented A. racemosus and G. officinalis in the drinking water compared to control group are in harmony with those reported by Saxena et al. (2010) who mentioned that chemical analysis of A. racemosus roots reveals the presence of steroidal saponins as Shatavarins IIV. Shatavarin isolated from roots of shatavari mimic female estrogen hormone and even replace it from its receptor. Also, Gupta and Shaw, (2011) hypothesized activity results from the hormone-like actions of these steroidal saponins. Another hypothesis declared that alcohol extract of Shatavari demonstrated estrogenic effects in genital organs and in mammary glands in rats with hyperplasia in alveolar tissues and acini and with increased milk production (Sharma and Bhatnagar, 2011). Although estrogens have a stimulating effect on the ductal epithelial cells, causing them to lengthen, their primary role seems to be the potentiation of PRL production (Mortel and Mehta, 2013). In addition to, Champavier et al. (2000); Gonzalez-Andres et al.(2004) indicated that several phytoestrogens have been isolated from methanol extracts of Goat's rue (G. officinalis) such as flavonol triglycosides, kaempferol, and quercetin. Also, Le Bail et al. (2000) reported that 2 phytoestrogens (sativan and medicarpin) isolated from leaves of G. officinalis. The antitumor activity of the aqueous extract from G. officinalis can be attributed to the alkaloids and flavonoids (phytoestrogens) found as major compounds in G. officinalis (Karakas et al., 2012). Gonzalez-Andres et al. (2004) reported the administration of phytoestrogens in low doses or foods containing G. officinalis could promote activation of some estrogen receptors in the animal. In addition, our previous research proved that antioxidant activity, phenols and flavonoids are present in water crude extracts of the aerial parts G. officinalis and A. racemosus roots (Hamed, 2016).

The effects of aqueous extract of A. racemosus and G. officinalis supplementation in drinking water on carcass traits and reproductive tract and some sex glands of rabbit does during first pregnancy are shown in Table 1. Supplementing the rabbit does with G. officianalis and A. racemosus was performed during the rearing period (from 10 weeks of age to first insemination) and extended continuously until slaughtering during pregnancy at first parity. Both concentrations of A. racemosus root increased ($P<0.01$) dressing percentage compared to the control and those of G. officinalis groups. However, A. racemosus at lower level (100 mg/kg b.w) showed higher ($P<0.01$) dressing percentage than A. racemosus at (200 mg/kg b.w). The relative weight of liver was increased ($P<0.01$) due to A. racemosus or G. officinalis administration compared to the control group, whereas the relative weight of spleen and kidney did not statistically differ among all experimental groups.

Data in Table 1 showed that absolute and relative weight of uterus included embryos did not significantly differ due to A. racemosus and G. officinalis supplementations compared to the control group. Whereas addition of A. racemosus and G. officinalis in the drinking water led to significant increase for absolute and

relative weights of paired ovary compared to control group. In addition, group supplemented with A. racemosus and G. officinalis at lower level significantly increased absolute weight of pituitary and mammary gland than to the control and A. racemosus at high level groups. However, differences between supplementations at high level of G. officinalis and other treatments were not statistically different for absolute weight of pituitary. High significant increase for relative weight of mammary gland was recorded in treated group with lower level of G. officinalis than to the other herbal groups. Addition of A. racemosus at high level significantly increased absolute and relative weight of brain compared to other experimental groups. These results are in agreement with those mentioned by Palep (2003) who reported the addition of A. racemosus containing preparations stimulate haemopoetic function and increase weight of ovaries and may enhance folliculogenesis, as evidenced by study of ovaries of immature female rats. Gopumadhavan et al. (2005) reported that, the energy source for the female reproductive system is estrogen dependent glycogen. Estrogen increases the glycogen content in the uterus and any decrease in uterine glycogen would directly implicate estrogen deficiency. Asparagus racemosus extract containing formulation was found to cause an increase in uterine weight and uterine glycogen without altering serum estrogen progesterone levels in immature rats as compared to ovariectomized rats used as control.

The histological examination of posterior portion of the mammary gland in all groups shows that it consisted of lobes containing numerous lobules. Each lobule consisted of two main tissues, stromal connective tissue and paranchymal tissue. The stroma is mainly fat and fibrous tissue located between mammary lobes and lobules to connect the paranchymal tissues, which were composed of two systems, the secretary system (Alveolar tissue) and the ductular system (collecting ducts) as shown in plate 1.

The effect of treatments on the histometric structure of the mammary gland focused on changes in surface area of lobes, lobules and alveolus. In addition, thickness of myo-epithelial layer and diameters of the mammary lobes and lobules were determined in rabbit does of each treatment group are presented in (Table 2) and illustrated in Plates 1 and 2. Supplementation with different concentrations of aqueous extract of G. officinalis and A. recemosus induced a significant increase in the surface area and diameter of lobes, lobules and surface area and numbers of alveolus compared to control. Moreover, the highest significant value of thickness of myo-epithelial layer and surface area of the mammary alveoli were shown form dose on G. officinalis supplemented with the highest dose compared with control group, but difference was not significant from the rest of groups.

The significant rapid mammary gland development for rabbit does treated with aqueous extract of A. racemosus and G. officinalis during rearing period and continuously during pregnancy may be related to the significant increase of estrogen and progesterone levels compared to control. This conclusion is keeping with those reported by; Kass et al. (2004) who indicated that many factors play a role in the development of breast milk. During early pregnancy, estrogen and progesterone develop key components of the breast tissue for lactation; estrogen stimulates milk duct development and progesterone forms lobules that are responsible for milk production. Prolactin is the predominant hormone that stimulates mammary glands; however, high progesterone and estrogen progesterone levels during pregnancy suppress prolactin's action on milk production during pregnancy. Additionally, prolactin and human chorionic

somatomammotropin stimulate the production of enzymes required for milk production. In addition, systemic administration of alcohol extract of A. racemosus in weaning rats increased weight of mammary glands, inhibited involution of lobulo alveolar tissue, and maintained milk secretion (Sabnis et al .,1968; Sahay and Pandey, 2003). A significant increase in milk yield has also been observed in pigs and goats after feeding with lactare (commercial herbal galactogogues with A. racemosus in its formulation) which also increased growth of the mammary glands, alveolar tissues, and acini (Narendranath et al., 1986). Other studies with alcohol extract of Shatavari (A.racemosus) demonstrated estrogenic effects in genital organs and in mammary glands in rats with hyperplasia in alveolar tissues and acini and with increased milk production (Sharma et al., 2011).

The influences of G .officianalis and A. racemosus in drinking water of rabbit does during the rearing period (from 10 weeks of age to the first insemination) and throughout pregnancy up to first 21 days of lactation during the first parity on milk yield ,litter size and litter weight of rabbit does are shown in Table 3. The herbal supplementations had no significant influence on the weights of rabbit does at mating and at birth. The total number of litter at birth among different experimental groups did not statistically differ. Whereas, number of kids born alive was significantly decreased in rabbit group received high level of aqueous extract of A. racemosus compared to low level and both levels of G. officinalis groups but not statistically changed with control group. The largest number of kits born alive at birth was recorded by both levels of G. officinalis compared to the highest level of A. racemosus and control groups. Also, the largest significant increase of kids of dead born was observed for high concentration of A. racemosus compared to low concentration group of A. racemosus and both levels of G. officinalis groups but not statistically changed with control group. Supplementation the rabbit does with G. officinalis at low level significantly increased litter size at 21 and 28 days of birth compared to high level of A. racemosus and control groups, but did not significantly differ compared to the other experimental groups. Both levels of G. officinalis significantly increased litter weight at birth, and at 21 and 28 days of birth compared to high level of A. racemosus and control groups but was not significantly differed compared to low level of A. racemosus. Also, supplementation the rabbit does with both levels of G. officinalis significantly increased daily milk yield at 7, 14 and 21 days compared to control one. Whereas, daily milk yield was not significantly affected by both levels of A. racemosus compared to control except that for the low level of A. racemosus at 7 days of birth at first parity.

Data of Tables 4 and 5 represent the effect of aqueous extract of G. officinalis and A. recemosus supplementation during the rearing period from 10 weeks of age to first insemination and only from birth up to first 21 day of lactation on productivity (litter size, litter weight and milk yield) of rabbit does for second and third parities. Data in table 4 showed that weight of rabbit does at mating and at birth was significantly increased only for group supplemented with high level of G .officinalis compared to those for low level. Total number of litter size and number of kids born alive at birth were increased ($p<0.0001$) for groups supplemented with higher levels of G. officinalis or A. recemosus than the lower levels and control groups. While, there were no statistical differences between groups with respect to dead litter size at birth. It is apparent from data of Tables 4 and 5 that groups of high doses of G. officinalis or A. recemosus were significantly increased compared to control with respect to litter size and weight at birth, at 21 and at 28 days of lactation.

Supplementation of both levels of A. racemosus and G. officinalis significantly increased daily milk yield at day 7 compared to control. Whereas, addition of G. officinalis or A. recemosus at higher levels significantly increase daily milk yield at 14 and 21 days than the control in the second and third parities (Table 5).

The results of the current study regarding the significant increase of daily milk yield (Tables 3 and 5), are in agreement with several researches on cows, sheep and rat. In this respect, Latvietis et al. (2002) indicated that lactogenic value has to be considered of G. officinalis which increase in milk yield and lactation persistency when included in a daily diet in cows. In addition Gonzalez-Andres et al. (2004) reported that lactogenic properties of G. officinalis were noticed in sheep at daily doses of 2 g of dry matter/kg body weight from the first month after lambing and during 60 days; the result was a 16.9% increase in total milk yield, without any signs of toxicity. Phytoestrogens administration with low doses or foods containing them could promote activation of some estrogen receptors in the animal and increase milk production. Several phytoestrogens have been isolated from methanol extracts of Goat's rue such as flavonol triglycosides, kaempferol, and quercetin (Champavier et al., 2000 ; Gonzalez-Andres et al., 2004).The role of flavonols like kaempferol and quercetin, phytosterols like diosgenin and phytochemicals with dopamine antagonistic activity like anethole, as active compounds in charge for galactogogue effect, is featured (Champavier et al., 2000; Peirs et al., 2006 and Paarakh, 2010).Also, Kahkeshani et al. (2015) indicated that total phenols, total flavonoids and the antioxidant effects is rich in G. officinalis. The positive impact on milk quantity and quality can be attributed to their active phytochemicals like phenols, flavonoids, saponins, terpenes and sterols. In addition Bailey et al. (2007) reported that Metformin was originally developed from the herb goat's rue (G. officinalis), which is known for its antidiabetic properties as well as its reputed ability to stimulate milk production and breast gland development. Several studies concluded that metformin is as effective and safe as insulin for the treatment of gestational diabetes (Gilbert et al., 2006). Farmers used G. officinalis as a lactation aid in Europe because of its observed effects on dairy cattle in the late 19th century (Abascal and Yarnell 2008). Another study indicated a lactogenic effect in the mothers of preterm infants (Castoldi et al. 2014).

With respect to milk yield increase by addition of aqueous extract of A. racemosus (Tables 5). Rajesh et al. (2010) indicated that female rats that received oral doses of milk decoction of A. racemosus (100mg/kg body weight) during their first lactation produced about 27% more milk than controls ($P<0{\cdot}05$). Pup weight gain was significantly higher than that in the control group. Aqueous decoction of A. racemosus in same dose produced only 6% more than control. Pandey et al. (2005) indicated that the root powder from A. racemosus oral administration in women in a double-blind randomized clinical trial has demonstrated a threefold increase in PRL level in subjects of the research group compared to the control group. Also, A. racemosus (shatavari) has galactagogue and mammogenic function through enhancing blood prolactin and cellular division in mammary gland to augment lactation and a lactogenic effect was reported in rats supplemented with the plant .However, in previous works authors did not observe any increase in PRL levels in A. racemosus treated females suffering from a secondary lactational failure (Sharma and Bhatnagar, 2011). In addition, Sharma and Sharma.(2013) indicated that Asparagus racemosus also supports deeper tissue and builds blood, helping in treating infertility, prevents miscarriage and acts as a post-partum tonic as it increases lactation,

regularizes the uterus and balances hormones, probably due to phytoestrogens. Kumar et al. (2014) concluded that pre-partum supplementation of A. racemosus significantly increased milk yield, colostrums total immunoglobulin and reduced total milk cholesterol, service period and service/conception in ensuing lactation.

These results demonstrate the superiority of the total and alive litter size at birth, litter size and weight at weaning (28 days of lactation) and daily milk yield at 14 and 21 days of lactation for group supplemented with A. racemosus at high level (200mg/kg BW) when added during rearing period and then from birth up to first 21 day of lactation (Table 4), but the same dose of A. racemosus when added continuously during pregnancy to lactation induced the lowest total and alive litter size at birth, litter size and weight at weaning and daily milk yield at 14 and 21 days of lactation. As a result of the high significant number of litter which born dead (stillborn) for first parity (Table 3). Also, it has been observed at slaughter the presence of the absorption of some embryos and noted aberrant embryo spacing within the uterine horns when the same dose of A. racemosus (200mg/kg BW) was added during pregnancy for first parity as shown in Plates 3 and 4.

Jetmalani, et al. (1967) indicated that root extracts of A. racemosus even at very high doses did not produce any abnormality in behaviour of rats and mice and the plant is safe during pregnancy and lactation. Also, Prabha et al. (2004) assessed the safety profile of A. racemosus by studying acute and chronic toxicity (1g/kg) on pre and post-natal developments in rats. Both studies indicated no changes in general behavior, gait, food and water intake body weight. Furthermore no changes in liver and renal function test parameters are reported. In addition to, Bhosale, et al. (2003) reported that, A. racemosus root extract has shown to treat pre-eclampsia associated with pregnancy. That regular use of this A. racemosus during antenatal period enhances the fetal wt. and foetal out come and decreases the incidence of perinatal deaths. These results are inconsistent with our findings when A. racemosus at high level was added during pregnancy for first parity (plate 1 and 2 respectively). In the current results we found increases ($p<0.0013$) the incidence of perinatal deaths (Table 3), the presence of the absorption of some embryos (Embryonic losses caused by failure of preimplantation development) and aberrant embryo spacing within the uterine horns. It may be related to imbalanced steroid hormones before implantation .As a result of the supplemented A. racemosus at high level during pregnancy, which significantly increased ovarian hormones compared to control group (figure 1). This possibility is consistent with previously report by Dey et al. (2004) who indicated that the implantation process requires precisely regulated ovarian hormones. In mice and rats, the hormonal environment in preimplantation uteri is progesterone primed, followed by a small surge of estrogen in the morning of Day 4 to prepare a receptive uterus for embryo implantation. The precise level of estrogen at this stage is critical because abnormally hypo or hyper estrogen level will both adversely affect uterine receptivity and decidualization (Das et al., 2009). The proper interaction of progesterone and estrogen would also be responsible for the accurate intrauterine embryo distribution and at the stage of embryonic implantation. It was reported by Greenwald (1957) that exogenous estrogen administration in rabbit while the embryos were in the uterus caused abnormal spacing of implantation sites, demonstrating imbalanced steroid hormones before implantation disrupted normal embryo spacing. Interestingly, another study in rat found elevated estrogen level and delayed/decreased progesterone level were associated with abnormal embryo spacing

and aggravated the effect of nicotine on disrupting embryo spacing (Yoshinaga et al., 1979).

IN CONCLUSION

water supplementations with aqueous extract from both concentrations of G. officinalis in the drinking water for rabbit does could be recommended to improve pubertal mammary gland development, increasing milk yield and enhancing productivity of rabbit does. In addition, supplementation with A. racemosus should be avoided in pregnant rabbit does. So, the feeds containing high doses of estrogen-like compounds (phytoestrogen) may be responsible "outbreaks" of infertility in rabbit farms. Based on previous results, any herbs can be potentially toxic if not used properly at proper dose, through specified route of administration. Standardization the methods and dose rate, long and short-term to detrimental the effects of these herbs on the metabolic rate of related body tissues should be done, are essential for the understanding of the use of these herbs.

Table (1): Effect of aqueous extract of Asparagus recemosus and Galega officinalis on carcass characteristics and reproductive organs of nulliparous V-line rabbit does (mean± SE)

Trait	Control	G. officinalis at		A. recemosus at		P value
		150 mg/kg BW	300 mg/kg BW	100mg/kg BW	200mg/kg BW	
Dressing (%)	49.0±1.15^{c}	48.46±0.37^{c}	48.47±1.03^{c}	57.78±0.94^{a}	52.9±0.72^{b}	P<0.0001
Liver (%)	2.93±0.02^{b}	3.61±0.07^{a}	3.68±0.005^{a}	3.55±0.32^{a}	3.91±0.16^{a}	P<0.0079
Spleen (%)	0.06±0.002	0.05±0.0	0.06±0.009	0.06±0.003	0.06±0.01	P<0.8431
Kidney (%)	0.52±0.003	0.57±0.001	0.61±0.02	0.56±0.05	0.65±0.05	P<0.1215
Wt of Pituitary (g)	0.022±0.001^{b}	0.028±0.0001^{a}	0.025±0.0^{ab}	0.027±0.001^{a}	0.02±0.001^{b}	P<0.0016
Wt of brain(mg)	6.12±0.30^{c}	6.10±0.15^{c}	7.79±0.11^{b}	7.34±0.76^{bc}	9.31±0.28^{a}	P<0.0017
Brain %	0.16±0.009^{c}	0.17±0.001^{c}	0.24±0.004^{b}	0.20±0.02^{bc}	0.28±0.02^{a}	P<0.0001
Wt uterus +embryos(g)	66.23±6.91	81.40±11.03	63.87±0.59	76.13±13	76.23±1.43	P<0.5445
Uterus +embryos %	1.54±0.18	2.10±0.27	1.87±0.04	2.09±0.39	2.28±0.01	P<0.6015
Paired ovary weight (g)	0.53±0.01^{b}	0.67±0.02^{a}	0.71±0.06^{a}	0.68±0.03^{a}	0.64±0.0^{a}	P<0.0088
Paired ovary %	0.015±0.0^{b}	0.019±0.0^{a}	0.02±0.001^{a}	0.019±0.0^{a}	0.02±0.0^{a}	P<0.0031
Paired mammary weight (g)	60.26±1.54^{c}	100.45±1.34^{a}	70.71±5.51^{b}	74.38±2.13^{b}	56.4±3.20^{c}	P<0.0001
Paired Mammary %	1.84±0.07^{b}	2.77±0.04^{a}	2.08±0.19^{b}	2.0±0.02^{b}	1.72±0.14^{b}	P<0.0001

^{a,b,c,d} means in the same row with different superscript are significantly different (P≤0.05).

Table (2): Effect of aqueous extract of Galega officinalis and Asparagus recemosus supplementation on some histometric characteristics of the mammary gland of rabbit does

Trait	Control	G. officinalis at		A. recemosus at		P value
		150 mg/kg BW	300 mg/kg BW	100mg/kg BW	200mg/kg BW	
The distance between lobes (µm)	12140±1485^{a}	50.2±720^{b}	52.5±673^{b}	52.0±673^{b}	26.9±673^{b}	P<0.0001
The distance between lobules (µm)	29.2±2.62^{a}	19.8±2.8^{b}	21.0±2.6^{b}	20.5±2.6^{b}	20.1±2.6^{b}	P<0.0834
Thickness (µm) of myo-epithelial	0.668±0.494^{b}	4.48±0.53^{a}	5.23±0.49^{a}	5.49±0.44^{a}	5.03±0.49^{a}	P<0.0001
Surface area(µm^2) of:						
Each lobe	9890±1865^{b}	74686±2238^{a}	77216±1713^{a}	63434±1814^{a}	75369±1880^{a}	P<0.0353
each lobule	4945±926^{b}	24895±7445^{a}	25439±5709^{a}	21145±6047^{ab}	25123±6226^{a}	P<0.0614
each alveolus	245±46^{c}	579±88^{ab}	638±127^{a}	316±61^{c}	347±52^{bc}	P<0.0068
Mammary diameter (µm) of:						
each lobe	279±20^{b}	409±66^{ab}	449±52^{ab}	540±74^{a}	399±62^{ab}	P<0.0450
Each lobule (alveoli)	93±6.7^{b}	136±22^{ab}	150±17^{ab}	180±24^{a}	133±21^{ab}	P<0.0449
Number of alveolus for each lobe	13.88±1.6^{c}	50.6±8.2^{ab}	53.3±5.3^{a}	36.9±3.3^{b}	41.5±4.5^{ab}	P<0.0001

^{a,b,c} means in the same row with different superscript are significantly different (P≤0.05)

Table (3): Effect of aqueous extract of Galega officinalis and Asparagus recemosus supplementation on Productivity of rabbit does for first parity (means ±S.E)

Trait		Control	G. officinalis at		A. recemosus at		P value
			150 mg/kgBW	300 mg/kgBW	100mg/kg BW	200mg/kg BW	
Weight of doe at(g)	mating	3519±85	3653±99	3501±85	3382±85	3541±92	P<0.4546
	birth	3303±96	3423±96	3301±96	3187±96	3405±104	P<0.5519
Litter size at birth	Live	4.00±0.90^{bc}	7.3±0.78^{a}	6.14±0.40^{a}	5.86±0.78^{ab}	2.2±0.84^{c}	P<0.0009
	Dead	2.29±0.58^{ab}	0.6±0.6^{c}	0.0±0.0^{c}	0.86±0.58^{bc}	3.83±0.62^{a}	P<0.0013
	Total	6.29±0.57	7.9±0.57	6.29±0.57	6.7±0.57	6.0±0.61	P<0.1964
Litter size at	21 day of birth	4.00±0.64^{bc}	6.3±0.59^{a}	5.6±0.59^{ab}	5.0±0.59^{abc}	2.0±0.90^{c}	P<0.0231
	28 day of birth	3.83±0.68^{bc}	6.1±0.63^{a}	5.6±0.63^{ab}	5.0±0.64^{abc}	2.0±0.98^{c}	P<0.0425
Litter weight (g) at	birth	307±29^{b}	460±28^{a}	424±26^{a}	374±27^{ab}	302±40^{b}	P<0.0036
	21 day of birth	1511±141^{bc}	2069±141^{a}	2096±130^{a}	1718±130^{ab}	1127±246^{bc}	P<0.0038
	28 day of birth	1813±231^{bc}	2512±184^{a}	2595±170^{a}	2281±170^{ab}	1573±319^{bc}	P<0.0125
Daily milk yield (g) at	7 day of birth	70±9^{b}	112±8^{a}	107±7^{a}	108±8^{a}	98±14^{ab}	P<0.0170
	14 day of birth	95±10^{b}	153±10^{a}	154±9^{a}	127±10^{ab}	105±17^{b}	P<0.0015
	21 day of birth	144±18^{b}	222±16^{a}	226±15^{a}	180±16^{ab}	148±28^{b}	P<0.0068

^{a,b,c} means in the same row with different superscript are significantly different (P≤0.05)

Table (4): Effect of aqueous extract of Galega officinalis and Asparagus recemosus supplementation on Productivity of rabbit does for second and third parity (means ±S.E)

Treatments	Dose mg/kg BW	Weight of does (g) at		Litter size at birth			Litter size at	
		mating	birth	Total	born alive	dead	21 days	28 days
Control	0	3699±54^{a}	3394±43^{ab}	7.50±0.54^{b}	7.07±0.55^{b}	0.43±0.29	5.57±0.33^{d}	5.57±0.33^{c}
Galega officinalis at	150	3459±63^{b}	3264±62^{bc}	8.20±0.45^{b}	8.13±0.42^{b}	0.07±0.06	7.5±0.39^{bc}	7.1±0.34^{b}
	300	3623±44^{a}	3502±52^{a}	10.9±0.42^{a}	10.4±0.36^{a}	0.43±0.17	8.4±0.32^{ab}	8.36±0.32^{a}
Asparagus racemosus at	100	3560±59^{ab}	3242±37^{c}	8.29±0.50^{b}	7.71±0.43^{b}	0.57±0.29	7.07±0.43^{c}	7.07±0.43^{b}
	200	3704±40^{a}	3504±45^{a}	10.6±0.40^{a}	10.3±0.43^{a}	0.36±0.17	8.64±0.27^{a}	8.57±0.31^{a}
P value teatments		P<0.0079	P<0.0003	P<0.0001	P<0.0001	P<0.4736	P<0.0001	P<0.0001
Between parity								
Second parity		3583±39	3379±39	8.60±0.36^{b}	8.31±0.38	0.31±0.14	7.14±0.31	7.03±0.31^{b}
Third parity		3630±33	3380±32	9.60±0.38^{a}	9.08±0.34	0.48±0.16	7.74±0.24	7.66±0.24^{a}
P value (parity)		P<0.3741	P<0.9694	P<0.0333	P<0.0643	P<0.4420	P<0.0608	P<0.0483
P value (treat*pariy)		P<0.4152	P<0.5899	P<0.9985	P<0.9666	P<0.9666	P<0.8265	P<0.8155

$^{a,b\ c}$ means in the same column having different letters are significantly different (P<0.05)

Table (5): Effect of aqueous extract of Galega officinalis and Asparagus recemosus supplementation on Productivity of rabbit does for second and third parity (means ±S.E)

Treatments	Dose mg/kg BW	Milk yield at				Litter weight at		
		7 days	14 days	21 days	28 days	Birth (alive)	21 days	28 days
Control	0	94±4^{b}	139±6^{c}	183±10^{c}	90±5.9^{c}	434±23^{c}	1724±78^{c}	2282±96^{c}
Galega officinalis at	150	127±6^{a}	176±7^{b}	239±10^{b}	117±7.8^{b}	523±33^{b}	2149±64^{b}	2752±114^{b}
	300	142±6^{a}	219±8^{a}	256±10^{ab}	155±12^{a}	634±25^{a}	2434±60^{a}	3310±63^{a}
Asparagus racemosus at	100	128±7^{a}	169±11^{b}	227±12^{b}	117±7.7^{b}	470±29^{bc}	2047±80^{b}	2643±118^{b}
	200	143±7^{a}	205±6^{a}	282±11^{a}	145±7.2^{a}	679±24^{a}	2590±99^{a}	3262±95^{a}
P value		P<0.0001	P<0001	P<0.0001	P<0.0003	P<0.0001	P<0.0001	P<0.0001
Between parity								
Second parity		125±4	176±7	227±9^{b}	125±7	515±22^{b}	2080±68^{b}	2715±93^{b}
Third parity		129±5	187±7	248±7^{a}	124±6	579±22^{a}	2299±67^{a}	2985±80^{a}
P value (parity)		P<0.4810	P<0.1747	P<0.0313	P<0.8755	P<0.0097	P<0.0015	P<0.0028
P value (treat*pariy)		P<0.8456	P<0.9276	P<0.8364	P<0.9999	P<0.9631	P<0.8065	P<0.9273

$^{a,b\ c}$ means in the same column having different letters are significantly different (P<0.05).

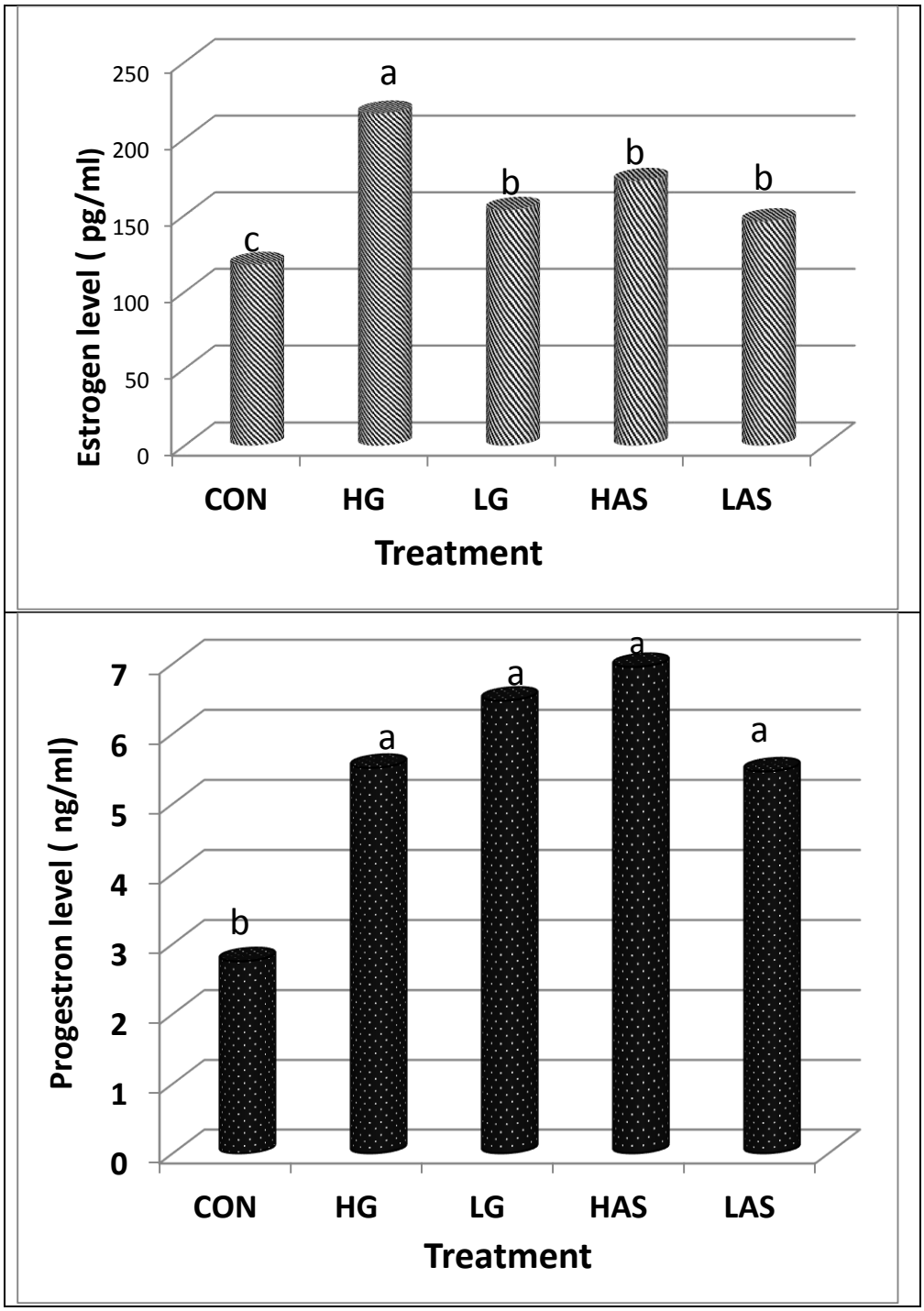
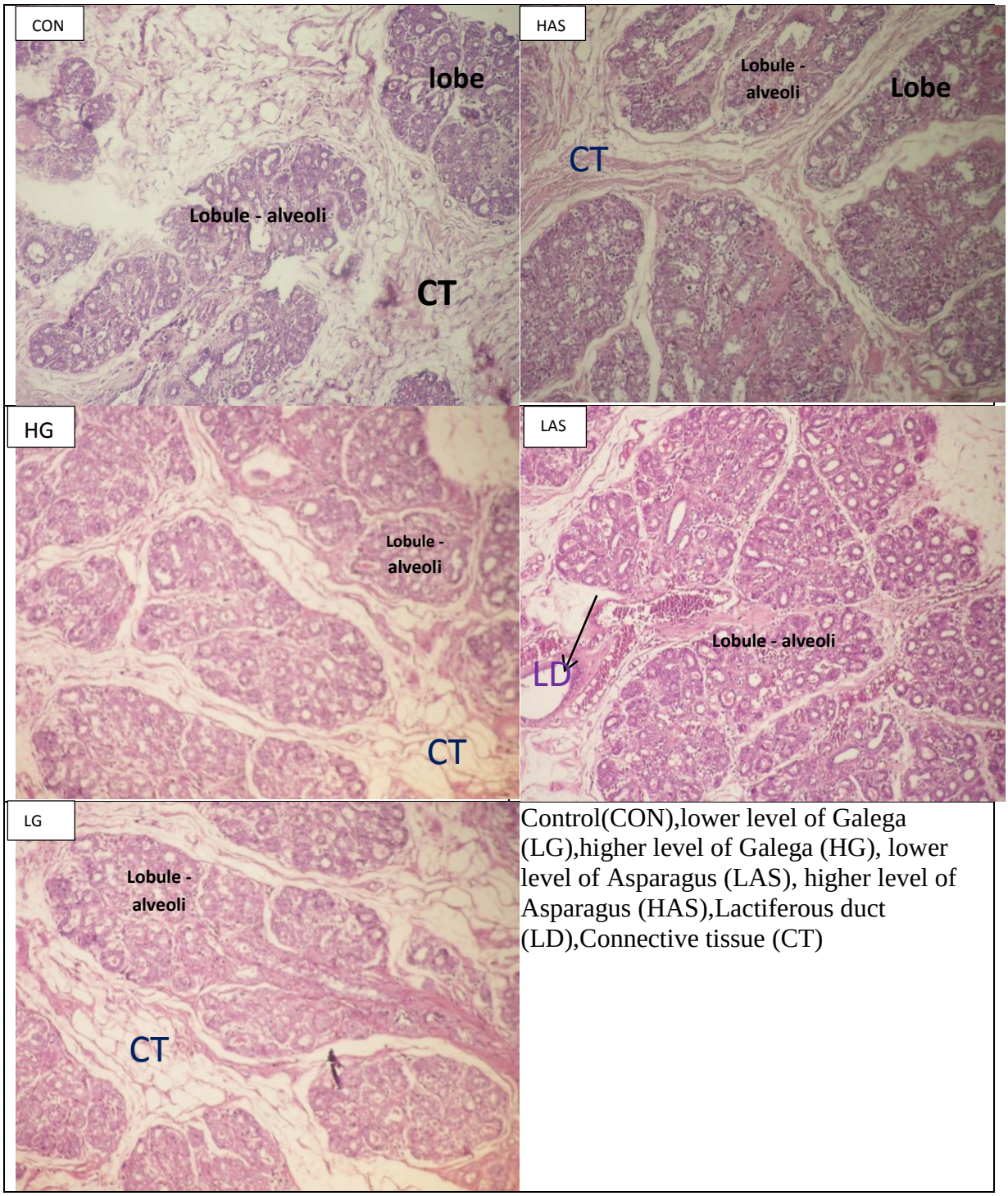
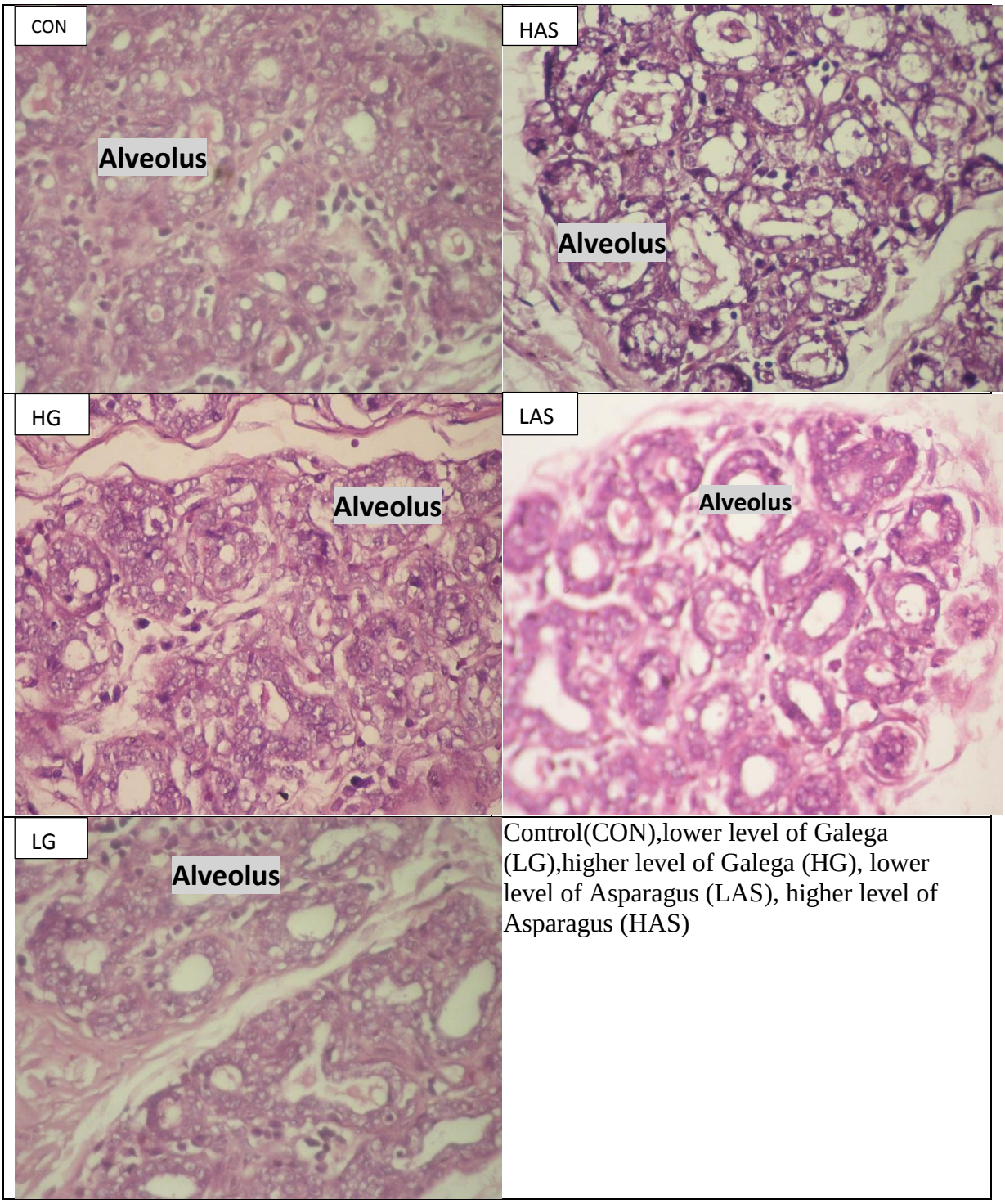


Fig (1): Effect of aqueous extract of Galega officinalis and Asparagus recemosus supplementation on ovarian hormones of rabbit does. Control(CON),lower level of Galega (LG),higher level of Galega (HG), lower level of Asparagus (LAS), higher level of Asparagus (HAS).



Control(CON),lower level of Galega (LG),higher level of Galega (HG), lower level of Asparagus (LAS), higher level of Asparagus (HAS),Lactiferous duct (LD),Connective tissue (CT)

Plate (1): Cross-section in mammary gland of rabbit does showing the effect of aqueous extract of Galega officinalis and Asparagus recemosus supplementation on more rapid pubertal mammary gland development during pregnant period.



Control(CON),lower level of Galega (LG),higher level of Galega (HG), lower level of Asparagus (LAS), higher level of Asparagus (HAS)

Plate(2): Cross-section in mammary gland of rabbit does showing the effect of aqueous extract of Galega officinalis and Asparagus recemosus supplementation on surface area of each alveolus, and density of alveolus during pregnant period.

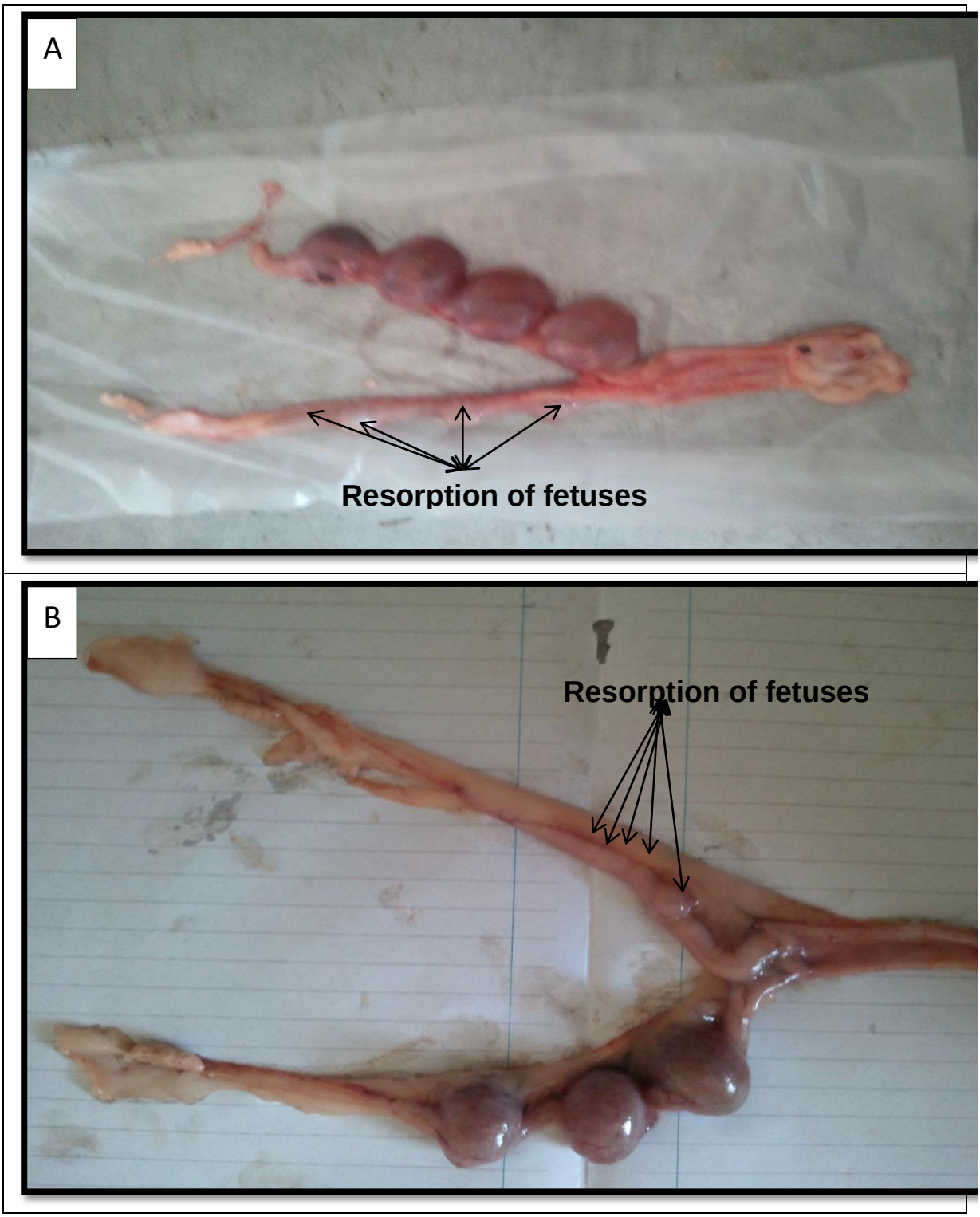


Plate (3): Reproductive tract of V-line rabbit does supplemented with aqueous extract of Asparagus recemosus at higher level (200 mg/kg B.W) during pregnant which effected on embryonic resorptions.

Plate (4): Reproductive tract of V-line rabbit does supplemented with aqueous extract of Asparagus recemosus at higher level (200 mg/kg B.W) during pregnancy

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الملخص العربى

تأثير إضافة المستخلص المائى للمدرة المخزنية والأسبرجس على تطورغدة الثدى وإدرار اللبن ومردود ذلك على القدرة الإنتاجية لإناث الأرانب

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قسم بحوث تربية الأرانب والرومى والطيور المائية- معهد بحوث الإنتاج الحيوانى- مركز البحوث الزراعية- الجيزة- مصر

أجريت هذة التجربة لتقييم مدى تأثير إضافة المستخلصات المائية لكل من نبات المدرة المخزنية (الجاليجا) وكذلك جذور نبات الهليون(الأسبرجس) فى مياة الشرب على النمو التطورى لغدة الثدى و إدرار اللبن ومردود ذلك على القدرة الإنتاجية لإناث الأرانب أثناء فترة التناسل.تم إضافة المستخلصات خلال فترة الرعاية وحتى التلقيح ، ثم إمتدت الإضافات بعد ذلك لثلاث بطون متتالية . أثناء فترة التناسل تم إضافة المستخلصات بصفة مستمرة خلال الحمل والرضاعة وذلك للبطن الأولى ، اما بالنسبة للبطن الثانية والثالثة فقد تم إضافة المستخلصات المائية خلال فترة الرضاعة فقط (حتى اليوم ال21 من الولادة) . خصص لهذة التجربة 60 انثى من سلالة الفى لاين عند عمر 10 اسابيع. قسمت الأرانب عشوائيا إلى خمسة مجموعات (12انثى لكل معاملة). المعاملة الأولى كنترول بدون إضافات، المعاملتان الثانية والثالثة اضيف إليهما المستخلص المائى لنبات المدرة المخزنية بمقدار 150و300 ملجرام من المستخلص الخام /كجم من وزن الجسم على التوالى . أما المعاملتان الرابعة والخامسة فأضيف اليهما المستخلص المائى لجذور الأسبرجس بمقدار 100و200 ملجرام من المستخلص الخام /كجم من وزن الجسم على التوالى.وقد وجد أن إضافة المستخلص المائى لنبات الجاليجا والأسبرجس أدت إلى زيادة معنوية في تركيز هرمونات المبيض فى سيرم الدم وكذلك زيادة معنوية للمساحة السطحية لحويصلات الثدى (mammary alveoli) مقارنة بالمجموعة المقارنة . مستخلص نبات الجاليجا عند كلا المستويين أدى إلى سرعة نمو وتطور غدة الثدى وزيادة إدرار اللبن وبالتالى تفوق للقدرة الإنتاجية للإناث عند إضافة المستخلص سواء خلال فترة الحمل والرضاعة أو عند الرضاعة فقط وذلك مقارنة بالكنترول . نفس النتائج السابق ذكرها تم الحصول عليها عند إضافة مستخلص نبات الأسبرجس أثناء فترة الرضاعة فقط ، ولكن عند إضافة نبات الأسبرجس عند المستوى العالى (200 ملجرام من المستخلص الخام /كجم من وزن الجسم) أثناء فترة الحمل فقد لوحظ عند التشريح للإناث أن هناك نسبة عالية من إرتشاف الأجنة وكذلك عدم إنتظام للاوضاع الطبيعية للأجنة داخل قرون الرحم مما أدى إلى زيادة معنوية فى نسبة النفوق للخلفة عند الولادة وكذلك نقص فى كمية اللبن خلال البطن الأولى.الخلاصة إضافة المستخلص المائى لنبات الجاليجا فى مياة الشرب للارانب قد يؤدى إلى سرعة نمو وتطور غدة الثدى وزيادة إدرار اللبن وبالتالى تفوق للقدرة الإنتاجية للإناث.بينما، يوصي بتجنب إضافة نبات الأسبرجس لإناث الأرانب أثناء فترة الحمل،حيث أن الأعلاف التي تحتوي علي جرعات عالية من مركبات الأستروجين (phytoestrogen) قد تكون مسئولة عن نقص الخصوبة في الأرانب.