The Effectiveness of Genistein on Early Puberty of the Arabi Female Lambs: The Concentration of Sex Hormones and Development of Reproductive Organs

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Abstract: This study was undertaken at special sheep farm in Missan province. The study began at the 1st of September 2016 and ended at the 29th of December 2016. Sixteen female Arabi lambs, with an average age 210±5 days, and average body weight (23.88) Kg, were randomly divided into four groups as follows:. T1 as a control group, T2 treated with a dose of 0.5g genistein.head\(^{-1}\), T3 treated with a dose of 1g genistein.head\(^{-1}\). T4 treated with a dose of 2g genistein.head\(^{-1}\). All treatments were given genistein orally three times a week to investigate the effect of genistein treatment on sex hormones and reproductive organ development. The results showed significant (P<0.05) increase in concentrations of sex hormones (FSH, LH and estrogen) in female lambs treated 1g genistein for three times a week compared with control, T2 and T4 during the whole study period. The weights of the uterus, right ovary and ovary duct length of T3 group increased significantly (P<0.05) in comparison with T1 group. There were no significant (P<0.05) differences observed between treated groups in left ovary weight. Treated of Arabi female lambs with different levels of genistein improved the development of uterus during puberty.

Keywords: Fertility traits, Polymorphism, sheep, TGF-B gene.

Introduction

Sheep production depends on the reproductive trails such as the function of ovaries, sex hormones secretion, the ability of ewes to mate, pregnant, lactating and caring their lambs. The attempt to improve the reproductive efficiency is the major step for farms, especially in countries depend mainly on agriculture (Ibarra et al., 2000). There are many ways to improve the reproductive efficiency, such as, artificial insemination, genetics, embryo transfer and hormone treatment (Patton, 2012).

Genistein is a phytoestrogen belong to isoflavones compounds, which derived and found in natural plant such as soya bean, alfalfa and clover (Elizabeth et al., 2007). Genistein may affect the chemical structure of muscle, bone, blood and milk for many
animals (King et al., 1998). The phytoestrogen is playing a role in regulating the reproductive system function because it has the similar action of natural estrogren in the body (Anderson et al., 2002).

Genistein can activate and attach with both Alpha and/or Beta estrogen receptors, in cells membrane (Kuipere et al., 1998; Clarke et al., 2010). Genistein had the same phenol ring that present in natural estrogren (Turner et al., 2007). Christos et al., (2006) reported that the phytoestrogen compounds could effect on reproductive tract through stimulating the hypothalamus gland to release the gonadotropin releasing hormone (GnRH). Izabela et al. (2013) suggested that genistein is hydrated by the rumen micro-organism to P.ethel phenol, which affect the activity of ovary and uterus. According to Griffith, (2012) who suggested that fed isoflavones or treatment female lambs with different levels of genistein led to stimulate the gonad glands and early puberty with low body weight.

The purpose of this study was to determine the effect of genistein treatment on sex hormone concentrations and reproductive organ development in Arabi female lambs.

Materials & Methods
This study was carried out in the a private field in Missan province. It began at the 1st of September 2016 and ended at the 29th of December 2016 Sixteen female Arabi lambs, with an average age 210 ±5 days, and average body weight 23.88 Kg, were randomly divided into four groups as follows:. T1 as a control group (without any dosage of genistein). T2 treated with 0.5 g genistein. head⁻¹ (Soy-Free Genistein product by the Sopbora Japonica Company as powder). T3 treated with 1g genistein.head⁻¹. T4 treated with 2g genistein.head⁻¹ orally. Genistein doses were given three times a week. All animals were fed 2% of body weight barley daily during morning and evening at 7 a.m. and 2 p.m. respectively. The amount of feed provided adjusted weekly according to the new weights. After 15 days of genistein treatment, the rams were introduced to detect 1st estrus. Blood samples were taken monthly to determine sex hormone levels. Three animals were slaughtered from each treatment after reaching the age of puberty to determine the weights of reproductive organs such as uterus, right ovary, left ovary and ovary duct length. Also, samples from uterus were taken and kept in plastic containers, including 10% formalin for 24 hours, and then transported to the laboratory of Veterinary College, University of Basrah to study the development of the uterus tissue according to method of Luna (1968) by using Microtome (made in China).

Ten ml of blood was collected monthly from all animals from the jugular vein and put in a plastic tube without anticoagulants. Blood samples were separated by centrifugation at 3000 rpm for 15 minutes. All serum samples were frozen under -20 oC until analysis time. FSH, LH and estrogen concentrations were determined by using a procedure associated with the kit of Monobind Inc. Company.

The obtained data were analyzed statistically using Completely Randomized Design (CRD). The significant differences were tested by Revised Least Significant Differences (RLSD) at the P<0.05 levels of significance by using the program of SPSS (2012).
Results

Table (1) explained the effect of genistein treatment on sex hormone concentrations in Arabi female lamb’s blood. The concentrations of FSH, LH and estrogen hormones of T3 increased significantly (P<0.05) compared with other groups during all months. On the other hand, no significant differences were presented between T2 and T4 especially during second and third months.

Table (1): The effect of different levels of genistein on sex hormone concentrations in serum of Arabi female lambs (Mean ± SE)*.

<table>
<thead>
<tr>
<th>Hormone</th>
<th>Months</th>
<th>Groups (Treatments)</th>
<th>T1</th>
<th>T2</th>
<th>T3</th>
<th>T4</th>
</tr>
</thead>
<tbody>
<tr>
<td>FSH (ng.ml⁻¹)</td>
<td>First</td>
<td>0.33c ±0.05</td>
<td>0.58b ±0.06</td>
<td>0.76b ±0.09</td>
<td>0.56b ±0.08</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Second</td>
<td>1.06b ±0.38</td>
<td>1.43b ±0.30</td>
<td>2.88a ±0.52</td>
<td>1.34b ±0.52</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Third</td>
<td>1.90b ±0.12</td>
<td>2.04ab ±0.53</td>
<td>2.99b ±0.43</td>
<td>2.29b ±0.42</td>
<td></td>
</tr>
<tr>
<td>Mean</td>
<td></td>
<td>1.12b ±0.18</td>
<td>1.35b ±0.21</td>
<td>2.21b ±0.22</td>
<td>1.40b ±0.86</td>
<td></td>
</tr>
<tr>
<td>LH (ng.ml⁻¹)</td>
<td>First</td>
<td>1.13c ±0.29</td>
<td>1.30b ±0.28</td>
<td>2.13b ±0.42</td>
<td>1.77ab ±0.24</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Second</td>
<td>2.66b ±0.46</td>
<td>2.96b ±0.47</td>
<td>3.38a ±0.65</td>
<td>3.53a ±0.61</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Third</td>
<td>2.69b ±0.46</td>
<td>3.65a ±0.72</td>
<td>4.11a ±0.54</td>
<td>3.61a ±0.11</td>
<td></td>
</tr>
<tr>
<td>Mean</td>
<td></td>
<td>2.16c ±0.40</td>
<td>2.63bc ±0.492</td>
<td>3.20b ±0.41</td>
<td>2.91b ±0.32</td>
<td></td>
</tr>
<tr>
<td>Estrogen (pg.ml⁻¹)</td>
<td>First</td>
<td>20.01b ±1.70</td>
<td>25.04a ±2.49</td>
<td>25.60a ±3.66</td>
<td>20.69b ±3.46</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Second</td>
<td>21.01b ±2.89</td>
<td>25.17b ±3.70</td>
<td>29.82a ±2.24</td>
<td>25.92ab ±3.60</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Third</td>
<td>25.53c ±2.57</td>
<td>28.32bc ±3.00</td>
<td>39.48a ±2.89</td>
<td>31.88b ±3.35</td>
<td></td>
</tr>
<tr>
<td>Mean</td>
<td></td>
<td>22.25c ±2.40</td>
<td>26.18b ±3.06</td>
<td>31.63a ±2.26</td>
<td>26.16b ±3.47</td>
<td></td>
</tr>
</tbody>
</table>

*Different small letter within each row mean significantly differ (p<0.05).

Table (2) exhibited the effect of different levels of genistein on some reproductive organs in Arabi female lambs. The weights of the uterus, right ovary and ovary duct length of T3 lambs increased significantly (P<0.05) in comparison with those of T1 group. While, there is no significant (P<0.05) differences were observed between treated groups in left ovary weight except in T3. However, the right ovary was higher in weight compared with the left one.

Table (2): The effect of different levels of genistein on some reproductive organ weights (puberty, uterus, right and left ovary) in Arabi female lambs (Mean ± SE)*.

<table>
<thead>
<tr>
<th>Trails</th>
<th>Groups</th>
<th>T1</th>
<th>T2</th>
<th>T3</th>
<th>T4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Puberty weight (kg)</td>
<td>3</td>
<td>33.97c ±2.09</td>
<td>35.41b ±1.96</td>
<td>38.12a ±0.94</td>
<td>37.08ab ±0.83</td>
</tr>
<tr>
<td>Uterus weight (g)</td>
<td>3</td>
<td>10.94b ±1.11</td>
<td>12.45b ±1.14</td>
<td>17.97a ±1.95</td>
<td>15.69a ±1.23</td>
</tr>
<tr>
<td>Right ovary weight (g)</td>
<td>3</td>
<td>0.49b ±0.01</td>
<td>0.52b ±0.02</td>
<td>0.65a ±0.01</td>
<td>0.55b ±0.01</td>
</tr>
<tr>
<td>Left ovary weight (g)</td>
<td>3</td>
<td>0.29b ±0.01</td>
<td>0.48a ±0.03</td>
<td>0.32b ±0.02</td>
<td>0.41a ±0.02</td>
</tr>
<tr>
<td>Ovary duct length (cm)</td>
<td>3</td>
<td>13.0b ±0.99</td>
<td>17.0a ±0.81</td>
<td>17.5b ±1.52</td>
<td>13.5b ±1.11</td>
</tr>
</tbody>
</table>

*Different small letter within each row mean significantly differ (p<0.05).

Plate (1) displayed a histological section of female uterus of control group (T1). The uterine gland glands were circular, small, non-mature, resided in limit areas of the uterus inlay and so far from the epithelium layer of the uterus cavity with a normal
number. Also, those glands covered by cells that did not arrive to the mucus secretion phase. While, the uterine gland of T2 animals were active, bigger, taller, diffusion on more places in uterus inlay and nearer to the epithelium layer of the uterus cavity compare with control group (Plate 2).

There is an increase in the number of the mucus glands in animals of T3 and T4 groups (Plate 3 & 4 respectively). Those glands seem to be diffused over all areas of uterus inlay and biggest, tallest, widest and more attachment with the surface of the uterus cavity in comparison with T1 and T2 groups.

Discussion

The increase in the concentration of FSH and LH may due to that genistein stimulate gonadotropin releasing hormone GnRH from hypothalamus gland or may stimulate
estrogen- receptors because of the genistein when binding with those receptors will active the cyclic protein kinase (AMP) (Skinner & Dufourny, 2005). This result agrees with the results of Wojcik et al., (2006). While, the increase of LH concentration of T3 group related to the effect of genistein (like estrogen) through the elevation of positive estradiol feedback (Baratta et al., 2001), because of the genistein has the same function of natural estrogen in the body (Turner et al., 2007), so, unlikely that genistein causes any negative feedback on LH secretion. Genistein has ability to conjure up and calling the GnRH, which led to increases the LH secretion during the high wave of LH in ewes (Romanowicz et al., 2004). This result was similar to that found by Polkowska et al. (2004). Furthermore, the isoflavones have an intimacy to attachment with estrogen receptors in both ovary and hypothalamus gland (Kuiper et al., 1998) and caused an effect on enzymatic estradiol production (Wojcik et al., 2006), this may be a reason for the elevation of estrogen concentration in the treated groups compared with control once. Our result agreed with the results of Griffith (2012) who found an increase in estrogen concentration after feeding female lamb's soya bean (isoflavone).

The weights of the uterus, right ovary and ovary duct length of T3 animals increased significantly (P<0.05) in comparison with the TI group (Table 2), may due to that of T3 animals have higher weight at puberty, which reflected positively on the general body growth including reproductive organs (Hafez & Hafez, 2000). Or may due to that the similarity between genistein and estrogen, because they had the same structure and functions, on the smooth uterus tissues through stimulating the receptors of reproductive organ cells membrane and share in synthesis of proteins thus play an important role in uterus gland growth (Buerj, 2014). Whereas, Clapper & Paulson (2015) reported another reason that phytoestrogen increase the weight of the smooth muscles especially those belongs to the uterus and ovaries. A similar result was also observed by Mustonen (2015) and Izabela et al. (2013), they were recorded an increase in uterus weight of animals treated with phytoestrogens. However, the right ovary was higher in weight compared with the left one, because of the right one is more active than left once (Lawson, 2011). T3 animals showed more development in the uterus gland as compared with the control group, may be due to the increase in puberty weight and estrogen concentration in T3 female lambs (Table 1), as known, estrogen plays important role in the growth and development female tract (Thibault et al., 1993).

Conclusions

From our results, we concluded that treating Arabi female lambs with 1g genistein.head⁻¹ orally at three times a week caused an increase in the puberty weight and concentrations of FSH, LH and estrogen hormone (shortening age at puberty). Also, treated genistein at dose 1g.head⁻¹ elevate the activation of reproductive organ such as the uterus and ovaries.

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Conflict of interest: The authors declare that they have no conflict of interest.

Ethical approval: all applicable national and international guidelines for the care and use of animals were followed.
References


