The administration’s effect of domestic soybean, lablab bean and lima bean content of genistein to improve the productivity of Bali cattle

A Fitriyah¹*, Isyaturriyadhah², Y Mariani¹, NMA Kartika¹, R Harmayani¹, and A Jamili³

¹Faculty of Animal Science, Universitas NahdlatulWathanMataram, Jl. Kaktus 1-3 Kota Mataram, NTB 83125, Indonesia
²Faculty of Agriculture, Universitas Muara Bungo, Kabupaten Bungo, Jl. Pendidikan, Sungai Binjai, Kabupaten Bungo, Jambi 37211, Indonesia
³Faculty of Agriculture, Universitas Nahdlatul Wathan Mataram, Jl. Kaktus 1-3 Kota Mataram, NTB 83125, Indonesia

E-mail:abyadulfitriyah@gmail.com

Abstract. Bali cattle plays a significant role as producers of food products for humans. Legumes are used in high-quality animal feed to increase productivity. This study analyzed the use of domestic Soybean (Glycine max), Lablab bean (Lablab purpureus), and Lima bean (Phaseolus lunatus) as the genistein in Bali cattle feed to shorten postpartum estrus. Different drying methods were applied by putting the ingredients in the oven at 55 °C and the freeze dryer at -40 °C. Further, genistein was detected using the Thin Layer Chromatography Method at UV 254 and 366 to determine the Rf value. The parameters measured include genistein detection and genistein identification as well as genistein content in legume straw. The data were analyzed descriptive using ANOVA and t-test. The result showed that only two samples adhered to the gel silica plate, including domestic soybean straw using the oven and freeze dryer (DSOD and DSFD), with 0.48 in DSOD and 0.51 in DSFD of Rf value, that is close to the genistein standard with the Rf value of 0.50 and purple colour as the colour of genistein standard. Moreover, the statistical analyses indicated significant differences in the genistein content of legumestraw between DSOD and DSFD (P<0.05), where DSOD=0.662g/100g and DSFD=1.770g/100g. The domestic soybean straw is regarded as a potential source of Bali cattle feed to improve productivity.

Keywords: Genistein, soybeans, lablab beans, lima beans, productivity and Bali Cattle

1. Introduction

Bali cattle plays essential roles as producers of services and products useful for human consumption and is a national asset in the agriculture sector; hence, their existence needs to be preserved to increase productivity. Meanwhile, several efforts have been carried out by the Government to increase domestic beef production, including the crossbreeding of local cattles with other cattle from overseas through insemination technology (Artificial Insemination) for the genetic improvement of animals.

The industry of Indonesian meat consists of domestic and imported products, accounting for around 70% of total consumption. Consequently, local production has not been able to meet the demand for beef; hence, the
country has constantly imported cattles and frozen beef for the past ten years. In July 2021, Indonesia imported approximately 3,500 tons worth $18 million from Brazil. Also, Australia currently supplies about half of its boxed meat imports, estimated at 28,500 tons by the end of July, plus 20,400 tons of meat offal. India supplies about 27 pc of buffalo, the United States about 9.5pc of meat, and New Zealand about 9pc of meat [1]. Moreover, this year Indonesia has allocated an import permit for 80,000 tons of meat from India [2], while the total value of red meat and livestock exports in fiscal 2020 was AU$1.2 billion from Australia [3], and animals worth $575 million were also imported in 2017 [4]. Nevertheless, the Indonesian Government has refused to increase the beef price instead of turning to other protein sources.

Increasing the domestic cattle population to increase sources of beef production is expected to reduce imports. Meanwhile, several efforts have been carried out to increase cattle population, such as improving productivity by selecting a stud [5]. However, the implementation has frequently failed. Therefore, there is a need to develop strategic methods to increase the cattle population by using feed quality. The criterion commonly used is the quality of sperm, including motility [6],[7]. Another attempt to increase the productivity and reproducibility of cattles is the Artificial Insemination strategy [8],[9], [10], but the motility of the spermatozoa determines the performance.

The activities to increase beef cattle production are limited due to the low reproductive performance of beef cattle. It occurs because of the calving interval of 20 months or more and the low birth rate of only 21% with calf mortality of 18% [11].

During this time, efforts have been overcome to the first post-calving estrus duration due to hormonal balance disorders by injecting Gonadotropin hormone-releasing hormone (GnRH) in post-calving cows that have long anestrus. However, GnRH injection at some sites is considered unsuitable. Due to the limited supply of GnRH, officers were unable to reach the farmer's location and the price was also too expensive. Therefore, the use of local ingredients that contain phytoestrogens which can stimulate GnRH becomes a solution.

Most of the phytoestrogen compounds are isoflavone derivatives, which are contained in soybean seeds or Glycine soja and other leguminous plants, such as lablab beans and lima beans [12]. A previous study stated that phytoestrogen acts as an estrogen in the body [13]. Moreover, it is a group of natural steroid hormones that play an important role in the growth and development of female sexual characteristics [14]. Furthermore, phytoestrogen works with progesterone to stop the ovulation process, which enhances the immediate occurrence of pregnancy [12].

Phytoestrogens are plant-derived dietary compounds, which are present in a wide variety of foods, especially in legumes [15]. Several legume plants are assumed to contain phytoestrogens such as soybeans, lablab beans, lima beans, and others.

The major class of phytoestrogens in soybean is isoflavones, however, genistein received the most attention. Moreover, the glycosylated form of genistein in soybeans products greater than 65% of the isoflavone content [12]. Meanwhile, the phytoestrogen content in soybeans can help meet the needs of estrogen in the body and become a natural hormone replacement food [16;17].

In addition to soybeans, Borlotti beans also have a nutritional content that is closer to peanuts and walnuts. The phytoestrogen content in peanuts is 104 mg/100 g [18], while walnuts have 127 mg/100 g [19]. Moreover, the nutritional content in jack beans is close to the contents in soybeans [20], therefore, it can be used as an alternative when there is a shortage of soybeans.

The analysis of isoflavones in biological samples is complicated due to the low efficiency of chromatographic separation and the lengthy sample preparation (8, 13-19). High-performance liquid chromatography (HPLC) together with electrochemistry, UV-vis diode-array detector (DAD), and/or detector mass spectrometry (MS) are among the most widely used methods for isoflavone determination. Meanwhile, the combination of effective chromatographic with adequate isolation/purification techniques is particularly suitable for quantitative analysis of compounds.

Therefore, this study aims to determine the content of isoflavones which are a source of phytoestrogens in soybeans, lablab beans, and lima beans with different drying methods as a genistein source of Bali cattle feed to improve the productivity.
2. Material and Methods
This study used Soybean (*Glycine max*), Lablab bean (*Lablab purpureus*), and Lima bean (*Phaseolus lunatus*) with different drying methods, including oven at 55 °C and freeze dryer at -40 °C. Furthermore, the genistein level was detected at UV 254 and UV 366 using Thin Layer Chromatography Method (TLC) to determine the Rf value, while the parameters measured include detection of genistein, identification of genistein and genistein content.

2.1. Ethical Approval
Since this study did not use live animals, ethical approval is not required.

2.2. Experimental Plants
The legumes were planted in polybags measuring 30 x 30 cm, with 2 to 3 seeds each. Hence, a total of 90 polybags were used as planting media. The plants were harvested after 110 days, followed by the oven and freeze-drying to determine the process with the highest levels of genistein.

2.3. Research Material
The straw of domestic Soybean (*Glycine max*), Lablab bean (*Lablab purpureus*), Lima bean (*Phaseolus lunatus*), and chemicals silica Gel F254, Genistein (standard), and aquadest were used in this study. While the equipment used includes a drying rack, oven, freeze dryer, blender, 10 ml test tube, filter paper, measuring cup, shaker, porcelain cup, electric heater, water bath, basin, analytical balance with a sensitivity of 0.1 mg, micropipette 0.5-10 µl, flacon, separating funnel, UV 254 and UV 366 lamps, and a set of densitometry tools.

2.4. Analysis of Genistein Content
Each 100 g of the samples was picked, washed, drained, air-dried, and dried in the oven at 55°C. Furthermore, the drying process was carried out at a low temperature of -4°C and made into powder. A total of 5 grams from each plant material was extracted with 50 ml of ethanol using a vortex and was filtered. The filtrate obtained was evaporated until it became thick, while 10 ml of hot distilled water was added and placed in a separating funnel, and 10 ml of technical hexane was added. After separation, the bottom layer was collected and shaken with 10 ml of ethyl acetate. Subsequently, the ethyl acetate layer was evaporated using a water heater until thick, dissolved with 2 ml of 95% ethanol, and the solution was prepared for isoflavones analysis.

The genistein content was determined using Thin Layer Chromatography (TLC) Densitometry, through quantitatively spotting the extract, followed by a standard genistein curve. Each test solution was spotted with 16 µl on a silica gel plate F254 (20 x 10 cm) with a distance of 1 cm between the spots. Furthermore, 2µl of genistein was also applied on the same plate as a comparison and was expanded to a height of 7 cm in a chromatographic vessel saturated with the mobile phase of toluene-acetate-formic acid with 7 – 3 – 0.1 v/v, upper phase. After the development was completed, the detection was carried out with UV 254 and UV 366 with a genistein comparison.

The isoflavone composition was analyzed by reversed-phase high-performance liquid chromatography (HPLC) [21], while the genistein content was determined by densitometry, through quantitative re-spotting of the extract, followed by the standard genistein curve with three replications.

2.5. Genistin Standard Curve Creation
The comparison solution was added to each of the extracts with varying concentrations of 0.5 µl; 1.0 µl; 1.5 µl; 2.0 µl on TLC silica gel plate F254. The solution was developed using toluene-acetate-formic acid as mobile phase with 7 – 3 – 0.1 v/v, upper phase. Subsequently, the genistein spots that appeared were measured by densitometry at a wavelength of 270 nm.

HPLC analysis has been tested as an alternative method to analyze genistein contents in legumes and other samples [22] but yields no difference from the previous manual methods used. It is carried out by pulverizing and vortexing samples for 1 min in 2.5 mL: 1: 1 hexane to methyl tert-butyl ether and the extraction solvent methylene chloride. Next, the samples were vortexed slowly for 15 min, followed by 10
min centrifugation at 3000 rpm to separate the aqueous and organic layers. Each piece was frozen at 80°C, and the organic layer was poured into a 10 mL glass conical screw cap and dried with nitrogen gas at 40°C.

Dry extracts and controls (i.e. genistein, daidzein, glycitin, and glycosides), were dissolved in 0.2 mL 1:1 mobile phase buffer A to mobile phase buffer B. Mobile phase buffer A consisting of 0.05% formic acid (HCO₂H) and 5 mM ammonium formate(NH₄HCO₂) in distilled water, while a mobile phase buffer B containing 0.05% formic acid and 5 mM ammonium formate in a ratio of 80:10:10, methanol acetonitrile with distilled water. The sample was vortexed vigorously for 5 min and centrifuged for 2 min at 1500 rpm to remove insoluble material, while the supernatant was removed and transferred to a 0.25 mL polypropylene injection vial with a cap for each chromatographic process. The area under the curve is compared with the standard to obtain purity.

2.6. Data Analysis
Data observation on detection of genistein, identification of genistein, and content of genistein in legume straw were analyzed descriptive and using analysis of variance (ANOVA) with the "t" test [23].

\[
t = \frac{(Q1 - Q2)}{\sqrt{\left[\frac{(sd1^2 / ns 1)}{1} + \frac{(sd2^2 / ns 2)}{2}\right]}}
\]

Q1 = The first (1st) Sample Mean
Q2 = The second (2nd) Sample Mean
sd1 = The first (1st) Sample Standard Deviation
sd2 = The second (2nd) Sample Standard Deviation
ns 1 = The first (1st) Sample size
ns 2 = The second (2nd) Sample size

3. Results and Discussions
3.1. Detection of Genistein Legume Straw
The detection samples of legumes straw at UV 254 and UV 366 against two (2) samples spotted on the gel silica plate of domestic soybeans straw using the oven and freeze dryer (DSOD and DSFD), shown in Table 1.

| No. | Samples                                      | Rf value | Colour |
|-----|----------------------------------------------|----------|--------|
| 1   | Lablab beans straw using oven dryer (LLSOD)  | 0.43     | Beige  |
| 2   | Lima beans straw using oven dryer (LSOD)     | 0.40     | Brown  |
| 3   | Domestic Soybeans straw using oven dryer (DSOD)| 0.48     | Violet |
| 4   | Genistein                                   | 0.50     | Purple |
| 5   | Domestic Soybeans straw using freezer dryer (DSFD)| 0.51     | Purple |
| 6   | Lima beans straw using freezer dryer (LSFD)  | 0.41     | Brown  |
| 7   | Lablab beans straw using freezer dryer (LLSFD) | 0.44     | Beige  |

Table 1 shows that samples using oven and freezer dryer of Domestic soybean Straw have Rf values of 0.48 and 0.51 with purple colour. These values are close to the genistein standard with an Rf value of 0.50 and a light purple colour. Based on the results, it was concluded that samples of domestic soybeans straw using the oven and freeze dryer contain genistein, while other samples with Rf values and colours not close to the standard might contain flavonoids, isoflavonoids, stilbenes or lignans.

The examples of dropping from concentrations of genistein standard, DSOD samples and DSFD samples used silent phase gel silica F254, with moving phase of toluene mixture-acetate ethyl-formic acid (7:3:0.1, v/v, above phase) and evaluated by ultraviolet light 356 and UV 254. The chromatogram of the dropped results is shown in Figure 1.
3.2. Identification of Genistein Legume Straw

Identification results of legume straw in six (6) samples are listed in Table 2.

Table 2. The identification of six (6) legumes straw samples.

| Samples                                           | Identification |
|---------------------------------------------------|----------------|
| 1. Domestic soybeans Straw using oven dryer (DSOD) | +              |
| 2. Domestic soybeans Straw using freezer dryer (DSFD) | +              |
| 3. Lima beans Straw using oven dryer (LSOD)        | -              |
| 4. Lima beans Straw using freezer dryer (LSFD)     | -              |
| 5. Lablab beans Straw using oven dryer (LLSOD)     | -              |
| 6. Lablab beans Straw using freezer dryer (LLSFD)  | -              |

Note: + genistein was detected  
- no genistein detected

Identification results of Genistein Legume Straw in table 2 are consistent with Table 1. Detection using ultraviolet light 254, UV 366 shows that only domestic soybean straw contain genistein, either in DSOD or DSFD from sixth dropped samples (DSOD, DSFD, LSOD, LSFD, LLSOD, and LLSFD). The domestic soybean straw (DSOD and DSFD) had the same dot level and colour as genistein, which was yellow purplish. This is in line with [28], which stated that the isoflavonoids or flavonoids content in domestic soybeans is relatively high and ranges from 2.0 – 4.0 mg/g.

Domestic soybeans contain several isoflavones. Hence, the flavonoid content, including quercetin, is relatively small [24], while the most significant components are daidzein and genistein [25]. Genistein in raw domestic soybeans is approximately 1.106 g/g, soy milk 30 µg/g protein, and tofu 209 µg/g protein [26], [27].

Besides that, four other samples contained the different dots and colours with genistein. The same dot and colour samples as genistein were oven dried domestic soybean straw (DSOD) and freeze-dried domestic soybean straw (DSFD). Based on the result of evaluation, it could be said that oven-dried domestic soybean straw and freeze-dried domestic soybean straw had genistein. To determine the content of genistein from the extract samples, the KLT Densitometry method was used for measuring the
extract samples which contain genistein quantitatively followed by the genistein standard curve.

3.3 Genistein content of legume straw

The domestic soybeans straw was tested for its genistein content, and the results are shown in tables 1 and 2. The Domestic Soybeans Straw’s samples content of genistein using oven dryer (DSOD) and freezer (DSFD) are shown in Table 3.

| Samples | Genistein detected (gram /100gram) |
|---------|-----------------------------------|
|         | DSOD     | DSFD     |
| 1       | 0.666    | 1.779    |
| 2       | 0.659    | 1.772    |
| 3       | 0.662    | 1.759    |
| Average | 0.662 ±0.006 | 1.770 ±0.004 |

Note: *) = significantly different (P<0.05)

Based on table 3, the genistein content in domestic soybean straw using two dryer methods (the oven and freeze dryer) showed a significant difference (P<0.05). The genistein content in domestic soybean straw was higher with the freeze dryer, namely 1.108 g, compared to the oven dryer. This is presumably caused by the amounts of substances that evaporate during oven drying at a high temperature of 55ºC. This shows that the drying process has a significant effect on the genistein content in domestic soybeans straw.

During the fermentation or non-fermentation procedures being processed, such as heating, isoflavone transforms, and by-products such as genistein and other flavonoids obtained in unequal amounts [29],[30].

The concentration of genistein in domestic soybeans straw as shown in Figure 1. High Performance Liquid Chromatography shows genistein’s presence as a peak with a retention time of 13.88 ± 2.57 min.

![Figure 1. HPLC Chromatogram obtained from domestic soybean straw extract using the freeze dryer (DSFD) showing Genistein (Rt = 13.88 ± 2.57).](chart1.png)

Figure 2 shows that some of the peaks overlap, while others indicate impurities of the sample or
contamination of the mobile phase, which contains a mixture of solvents used to run the chromatography. However, only one peak was read, namely at 3215 mAU*S, the height of 13.88 mAU, and the resolution of 1.55, while others were not detected. This information is shown in the chromatogram, but the peak showing a change in area and height corresponds to genistein in the latter case.

These results are in line with [31], which stated that High-Performance Liquid chromatograms showed the presence of genistein as a peak with a retention time of 25.50± 0.02 min in vitro cultured tissues and two (2) significant isoflavones in S.junceum tissues during various phases of micropropagation, including genistein (4,7-dihydroxy isoflavones).

3.4 The growth of legumes at harvest stages I, II, III and IV

The growth of legumes, including Soybean, Lablab bean, and Lima bean, increased weekly. In the first week, the plants experienced slightly slower growth than the second and third, but the plant height measurement in this study began in the fourth week and above towards the harvest period to analyze its genistein content.

The data on the average of plant growth of legumes per week, shown in Figure 2.

![Figure 3: The average weekly growth of legume](image)

Meanwhile, the plant height (cm) measured from the base of the stem to the highest leaf. Based on Figure 3, the average increase in plants height showed a significant difference (P<0.05). The average increase between the fourth and seventh weeks ranged from 4.29 cm on domestic soybeans, 14.43 cm on Lablabbeans, and 8.24 cm on Lima beans indicates that Lablab bean plants have the highest plant growth rate compared to other legumes, but this does not affect the content of genistein, the highest genistein content found in domestic soybeans (Table 3).

[32] reported that all types of flavonoids, including genistein, hesperetin, and naringenin, did not significantly correlate with plant growth in legumes. However, this is different from [33], which found a high correlation between total flavonoid content and antioxidant activity in all growth phases. Furthermore, plant productivity and soil nutrient concentration positively correlate with the process of grassland degradation [34].
4. Conclusion
Based on the results, domestic soybean straw contains high genistein, which is close to the Rf value and colour of the standard of genistein, with values of 0.662g/100g in DSOD and 1.770g/100g in DSFD. Therefore, domestic soybean straw is potentially a source of genistein in animal feed to improve the cattle productivity.

5. Authors’ Contributions
Conceptualization and manuscript revision: A Fitriyah, and Isyaturriyadhah
Experimental conduct and data analysis: Y Mariani and NMA Kartika
Validation acquisition of data: R Harmayani and A Jamili
Investigation and manuscript writing: A Fitriyah
Supervision: Isyaturriyadhah and A. Fitriyah. All the authors have read and approved the final version of this manuscript.

6. Conflict of Interest
The authors state that there are no financial or personal relationships with other persons or organizations that might improperly influence the work of this study and no other professional or personal interests in the products, services and companies that might influence the content of the manuscript.

7. Funding
The Indonesian Government financially supported this research through the Ministry of Education, Research, and Technology with the research scheme: PenelitianKompetitif Nasional, PenelitianTerapan with contract No. 1961/LL8/KM/2021, 001/LPPM/UNW/VII/2021.

References
[1] Central B 2021 Indonesia halts beef imports from Brazil (Beef Central: Nascon Media Pty Ltd)
[2] Fitzgerald M BaD 2021 Indonesia suspends imports of Indian buffalo meat due to COVID-19 concerns (N.C. Hour, Editor: NEWS ABC RURAL)
[3] Australia mMaL 2020 Indonesia, in Market Snapshot Beef &Sheepmeat (MLA Industry Insights)
[4] Indoniesia A 2021 Agribusiness to Indonesia Export snapshot, in Export markets - Indonesia (Australian Trade and Investment Commission)
[5] Schöpke K and Swalve HH 2016 Review: Opportunities and challenges for small populations of dairy cow in the era of genomics Animal.101050-60
[6] Fraser L et al. 2020 Transcriptome analysis of boar spermatozoa with different freezability using RNA-Seq Theriogenology142400-413
[7] GhafarizadehAA et al 2021 The effect of vitamin E on sperm motility and viability in asthenotatorazoospermic men: In vitro study Andrologia53e13891
[8] Neglia G et al. 2020 Reproductive management in buffalo by artificial insemination Theriogenology.150166-172
[9] Baruselli PS, et al. 2018 Review: Using artificial insemination v. natural service in beef herds. Animal, 12(s1):s45-s52.
[10] Fitriyah AS, Harianto H and Said DO 2016 Motility spermatozoa of the cow after given crude tannin supplement, in the 3rd Animal Production International Seminarthe 3rd ASEAN Regional Conference on Animal Production, 3rd APIS & 3rd ARCAP – 2016 (Malang, Indonesia: Brawijaya University Press)
[11] Fitriyah AS, Harianto H and Said DO 2017 Improvement of sperm quality of Bali cattle by supplementation of crude tannin in the semen, in the 1st International Conference on Tropical Agriculture, (Bulaksumur, Yogyakarta, Indonesia: Springer link: 321-327)

[12] Jefferson WN et al. 2007 Disruption of the female reproductive system by the phytoestrogen genistein. Reprod Toxico23(3):308-316.

[13] Ko S H, & Kim H S.2020 Menopause-Associated Lipid Metabolic Disorders and Foods Beneficial for Postmenopausal Women. Nutrients. 12(1)

[14] Jennings K.J &Leea L.D. 2020 Neural and Hormonal Control of Sexual Behavior. Endocrinology 10 150-161.

[15] Wang PP et al. 2017 Comparative Structural Characterization of Spiral Dextrin Inclusion Complexes with Vitamin E or Soy Isoflavone. J Agric Food Chem. 65(39):8744-8753.

[16] Veremeichik GN et al. 2021 Isoflavonoid biosynthesis in cultivated and wild soybeans grown in the field under adverse climate conditions. Food Chem 1 342:349

[17] Sugiyama A et al. 2017 Synthesis and Secretion of Isoflavones by Field-Grown Soybean. Plant Cell Physiol. 58 1594-1600

[18] Morris JB 2009 Morphological and Reproductive Characterization in Hyacinth Bean, Lablab Purpureus (L.) Sweet Germplasm with Clinically Proven Nutraceutical and Pharmaceutical Traits for Use as a Medicinal Food. Journal of Dietary Supplements. 6 263-279

[19] Tiro BMW, &BedingdPA. 2018 The use of hay to feed the cow cruciferous soybean simmentalongole descent .JurnalIlmiah INOVAS. 18 23-28

[20] Lucia Margarita PH 2020 Starch Digestion Enhances Bioaccessibility of Anti-Inflammatory Polyphenols from Borlotti Beans (Phaseolus vulgaris). Nutrients. 12 150-156.

[21] Ohno Y et al. 2006 Effect of lectins on the transport of food factors in caco-2 cell monolayers J Agric Food Chem. 54:458-53

[22] Thomas BF et al. 2001 Quantitative analysis of the principle soy isoflavones genistein, daidzein and glycitein, and their primary conjugated metabolites in human plasma and urine using reversed-phase high-performance liquid chromatography with ultraviolet detection J Chromatogr B Biomed Sci Appl760:191-205

[23] Thakur M 2020 t-Test Formula EDUCBA

[24] Arinanti 2018 The potential of natural antioxidant compounds on various types of beans IlmuGizi Indonesia.01134

[25] EndangYuniastuti SS,RhiziaSyifa F andMarshelina Noor ID 2020 Diversity of White pigeon pea [cajanuscajan (l.) mill sp.] in Wonogiri district, Central Java Buletin Plasma Nutfah.2651-62

[26] Vo TT et al. 2020 Effects of Cajanus cajan (L.) mill sp. roots extracts on the antioxidant and anti-inflammatory activities Chin J Physiol. 63137-148

[27] Wu GY et al. 2019 Prenylated stilbenes and flavonoids from the leaves of Cajanus cajan. Chin J Nat Med. 17(5):381-386.

[28] Lu Y et al. 2018 Dietary domestic soybeans isoflavones in Alzheimer's disease prevention Asia Pac J Clin Nutr.2746-954

[29] Allred CDet al. 2001 Soy diets containing varying amounts of genistein stimulate the growth of estrogen-dependent (MCF-7) tumours in a dose-dependent manner Cancer Res.615045

[30] Fuchs D et al. 2006 Soy extract has different effects compared with the isolated isoflavones on the proteome of homocysteine-stressed endothelial cells Mol Nutr Food Res50:58-69

[31] Clematis F et al. 2014 Endogenous isoflavone methylation correlates with the in vitro rooting phases of Spartiumjuncceum L. (Leguminosae). J. Plant Physiol.1711267-75

[32] Yingchao Liu XY, Jingxiu Xiao, Li Tang and Yi Zheng 2019 Interactive influences of intercropping by nitrogen on flavonoid exudation and nodulation in faba beans Scientific Reports nature research. 94818

[33] Kim MA and Kim MJ 2020 Isoflavone profiles and antioxidant properties in different parts of domestic soybeans sprout J Food Sci.85689-695
[34] Xu HP et al. 2019 Responses of plant productivity and soil nutrient concentrations to different alpine grassland degradation levels *Environ Monit Assess.* **191**678
