Effects of bovine somatotropin on milk yield, mammary gland weight and histology in West African Dwarf goats

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SUMMARY

Twenty-four lactating West African Dwarf (WAD) goats were used to study the effects of bovine somatotropin (bST) on total milk yield (TMY), mammary gland weight and histology. The goats were equally assigned into four treatment groups, comprised six does each. The first group (control, T0) received no bST while the other groups received bST (T1, 20 mg; T2, 40 mg; T3, 60 mg) injected at 2-week intervals commencing from the 7th week postpartum for 6 weeks. The does were milked-dry twice daily by 7:30 a.m. and 7:30 p.m. respectively for 42 days commencing from the 7th to the 12th week of lactation. The yields were added to determine the daily milk yield (DMY) and the TMY was determined by cumulative daily milk yields for 42 days. At the end of twelfth week, twelve does (three each treatment) were milked-dry by hand, slaughtered and their udders were excised. Udder weight (UW), Mammary gland weight (MGW) and volume (MGV) were determined. The effects of bST on mammary gland histology were investigated. The analyzed parameters included number of alveoli lobules (ALD), lactating alveoli (LA), regressing alveoli (RA), total alveoli (TA), alveolar corpora amylacea (ACA), interstitial corpora amylacea (ICA), total corpora amylacea (TCA), alveolar ductular diameter (ALD), epithelial luminal diameter (ELD) and epithelial height (EH). TMY was higher in treated goats and significantly (p<0.05) increased with increased doses of bST. UW, MGW and MGV were higher in treated than control goats suggesting that with advance lactation, bST administration maintains milk secreting cells. Treatment of lactating goats with bST significantly (p<0.05) increased the number of LA and significantly (p<0.05) reduced the number of RA, ACA and ICA. ALD, ELD and EH increased with increased doses of bST. ALD and ELD were significantly (p<0.05) larger and EH was significantly (p<0.05) higher in treated than control goats. Therefore, our findings suggest that the administration of bST to lactating WAD goats after peak of lactation appears to change mammary gland activity increasing milk yield. This is related to the maintained mammary glandular tissue weight resulting from increased lactating alveoli.

Efecto de la somatotropina bovina sobre producción de leche, peso e histología de la glándula mamaria en cabras Enana Africanas Occidentales

RESUMEN

Se emplearon 24 cabras West African Dwarf (WAD) en lactación para estudiar los efectos de la somatotropina bovina (bST) sobre el rendimiento total de leche (TMY) e histología y peso de la glándula mamaria. Las cabras fueron asignadas a cuatro tratamientos en grupos compuestos por 6 hembras. El primer grupo (control, T0) no recibió bST mientras que los otros grupos si la recibieron (T1, 20 mg; T2 40 mg; T3, 60 mg) mediante inyección durante seis semanas, a intervalos de 2 semanas, comenzando a partir de la 7ª semana postparto. Las hembras fueron ordeñadas totalmente 2 veces al día a las 7:30 a.m. y a las 7:30 p.m. durante 42 días, comenzando desde la 7ª semana de lactación. Los rendimientos fueron sumados para determinar el rendimiento diario de leche (DMY); la TMY fue determinado acumulando los rendimientos diarios de leche durante 42 días. Al final de la semana número 12, doce hembras (3 de cada tratamiento) fueron ordeñadas completamente a mano, sacrificadas y las ubres desecadas. Se determinó también el peso de la ubre (UW), el peso de la glándula mamaria (MGW) y su volumen (MGV). Los efectos de la bST sobre la histología de la glándula mamaria se estudiaron en relación al número de lábulos alveolares (ALN), alveolos lactantes (LA), alveolos en regresión (RA), total de alveolos (TA), cuerpos amílacos alveolares (ACA), cuerpos amílacos intersticiales (ICA), total de cuerpos amílacos (TCA), diámetro ductal del alveolo (ALD), diámetro luminal del epitelio (ELD) y la altura del epitelio (EH). El TMY fue mayor en las cabras tratadas y aumentó (p<0.05) al hacerlo las dosis de bST. UW, MGW y MGV fueron más altos en las cabras tratadas que en los controles.
In view of the facts that mammary regression occurs after peak of lactation which is accompanied with decline in milk yield and deterioration in milk quality in advanced stages of lactation. Therefore, bST administration could be beneficial in modulating the rate of mammary gland involution in West African Dwarf (WAD) goat that is one among the most widely distributed dairy goat breed (FAO, 2016) but has low selection intensity, with fairly short lactation period where little information is available on the effects of bST treatment and improvement for dairying. In order to have a better understanding of how bST exerts its effects on mammary gland, we studied the effects of bST on total milk yield, mammary gland weight and histology in lactating WAD goats.

MATERIALS AND METHODS

DESCRIPTION OF THE EXPERIMENTAL SITE

The experiment was conducted at the goat unit of the College of Animal Science and Livestock Production Teaching and Research Farm, Federal University of Agriculture, Abeokuta. It falls within the Rain Forest Vegetation zone of South-Western Nigeria at latitude 7°13’ 49.46”N, longitude 3°26’ 11.98”E (Google Earth, 2015) and altitude of 76 meters above sea level. The climate is humid with a mean annual rainfall of 1037 mm. The annual mean temperature and humidity are 34.7 °C and 82 % respectively.

EXPERIMENTAL ANIMALS AND THEIR MANAGEMENT

All experimental procedures including use of animals were performed according to approval granted by the Research Supervisory Committee of Department of Animal Physiology, College of Animal Science and Livestock Production, Federal University of Agriculture, Abeokuta, Nigeria.

Twenty four lactating West African Dwarf lactating does of first and second parity with ages between 1.5 and 3 years and whose body weights ranged from 11 – 16 kg, were selected from existing flock on the farm and used for the experiment. The goats were housed in cross-ventilated pens with slatted floors and water given ad libitum. The goats were under intensive management system with zero grazing (‘cut and feed’) and 0.3 kg per animal day concentrate feed supplement, consisting of about 17 % crude protein, 11.56 % crude fibre, 4.68 % ether extract and 8.69 MJ/kg metabolisable energy was fed to the animals. Panicum maximum grass was chopped and 1.0 kg per animal per day was fed to the goats. The proximate composition of the grass consisted of 5.37% of crude protein, 33.35% of crude fibre, 66.31% of neutral detergent fibre, 42.79% acid detergent fibre and 19.71 % of acid detergent lignin respectively.

EXPERIMENTAL PROCEDURE

Twenty four lactating does were divided equally into four treatment groups, each group comprising of six does with first and second parity with liveweight and daily milk yield equalized among treatment groups. The first group, which was the control (T1) received no bovine somatotropin injection while the
remaining three groups received bovine somatotropin injections at different doses: 20 mg (T₁), 40 mg (T₂) and 60 mg (T₃). The bovine somatotropin, Lactotropina MR, division Sanidad Animal, Eli Lilly CO., Mexico is in a sustained-release formulation for 14 days interval.

The does were given three injections of bST spaced 2 weeks apart, i.e. at the onset of weeks 7, 9 and 11 of lactation. The doses of bST were selected based on the preliminary results obtained on the study on milk yield and composition of goats treated with bovine somatotropin in Egypt by Sallam et al. (2005).

**DATA COLLECTION**

**TOTAL MILK YIELD**

The animals were milked-dry twice daily in the morning and evening at 7:30 a.m. and 7:30 p.m. respectively. The a.m. and p.m. yields were added to determine the daily milk yield (DMY). The does were milked for 42 days commencing from the 7th to the 12th week of lactation. The total milk yield was determined by cumulative daily milk yields for 42 days.

**MAMMARY GLAND WEIGHT AND VOLUME AND HISTOLOGICAL PARAMETERS**

At the end of 12th week of lactation, 12 does consisting of 3 does per treatment were selected from the 24 does, milked-dry and subsequently slaughtered and their udders excised for the study on mammary gland traits and histological parameters. The udders excised were weighed to get udder weight (UDW) and there after trimmed of extra-parenchymal tissue (skin, teat and subcutaneous fat) and weighed by a sensitive electronic weighing scale with maximum capacity of 210 g and readability of 0.0001 g (Adventurer™, OHAUS Corp, Pime Brook NJ, USA) to get mammary gland weight (MGW). Mammary gland volume (MGV) was determined by Archimedes’s principle (Linzell, 1966). The udders were separated into right and left halves and mammary tissue samples taken medially from each half for histological analysis. The permanent histological slide of mammary tissue samples were produced following hematoxylin and eosin-staining technique as reported by Baldi et al. (2002).

The histological parameters evaluated through the permanent histological slide included number of alveolar lobules (ALN), number of lactating alveoli (LA), number of regressing alveoli (RA), total alveoli (TA), alveolar corpora amylacea (ACA), interstitial corpora amylacea (ICA), total corpora amylacea (TCA), alveolar ductular diameter (ADD), epithelial luminal diameter (ELD) and epithelial height (EH). The TA was counted on hematoxylin and eosin-stained sections. The numbers and positions of CA were estimated on sections stained with Congo red (Highman technique). The CA within the lumen of the alveolus was classified as ACA, and those in interalveolar connective tissue were classified as ICA. Alveoli and CA were quantified with the aid of a grid placed in the eyepiece. For each animal, 2 independent tissue blocks per doe were examined: a block from each gland (left and right). Two sections per block and five random fields per section were examined and evaluated at × 250 magnification. Therefore, 20 fields per doe, 60 fields per treatment group and 240 fields for the entire 12 does were evaluated and examined. The numbers of TA, LA, and RA were counted in each field, selecting alveolar sections in which most secretory cells presented sagittal section. The CA was counted in the same fields; for each field the number of TCA, ACA, and ICA were counted. Two independent observers performed counts.

ADD, ELD and EH were also evaluated using the same number of sections and fields stated for alveoli and CA. The permanent slides were examined under the microscope with calibrated eyepiece micrometer (Graticule Ltd., Tonbridge, Kent, England).

**STATISTICAL ANALYSIS**

Data generated on total milk yield, mammary gland weight and volume and histological parameters were subjected to analysis of variance (ANOVA) in 4 × 2 factorial design using the Systat Analytical Computer Package, version 5.02 (SYSTAT, 1992). The statistical model used was:

\[ Y_{ijk} = \mu + A_i + B_j + A^*_B_{ij} + \epsilon_{ijk} \]

Where:

\( Y_{ijk} \) = Trait of interest;

\( A_i \) = Fixed effect of i\textsuperscript{th} bST dose (i = 0, 20, 40 & 60 mg);

\( B_j \) = Fixed effect of k\textsuperscript{th} parity (k = 1\textsuperscript{st} & 2\textsuperscript{nd});

\( A^*_B_{ij} \) = Interaction between i\textsuperscript{th} bST dose and j\textsuperscript{th} parity of does;

\( \epsilon_{ijk} \) = Random error associated with each record.

The package analyzed the main effects of bST treatment and parity on mammary gland traits and histological parameters examined but failed for their interaction. This could be due to the small number of does per group (n= 3) in the experiment. Tukey’s Honest Significant Difference (HSD) was used to separate means where significant differences exist.

**RESULTS AND DISCUSSION**

Total milk yield, udder weight and mammary gland weight were significantly (p<0.05) influenced by bovine somatotropin administration and parity except for mammary gland volume that was not significantly (p>0.05) affected by bovine somatotropin administr-
tion and parity. Total milk yield increased with increased dose of bovine somatotropin and T₁, T₂, and T₃ exceeded T₀ by 50.36, 68.00 and 71.16% respectively (table I). The result of the present study could be attributed to the galactogenic activity of the growth hormone used resulting to proliferation of milk secreting cells for milk production which was earlier reported by James et al. (2010). Udder weight and mammary gland weight significantly (p<0.05) increased consistently with increasing doses of bovine somatotropin and increasing parity (table I). West African Dwarf does administered bovine somatotropin and those with second parity have heavier udder and mammary glands compared with the control. The heavier udder weight and mammary gland weight observed in bovine somatotropin-treated does compared with the control showed the mammogenic and galactopoietic ability of bovine somatotropin. Sejrsen et al. (1999) asserted that administration of growth hormone in mid-lactation caused an increase in the quantity of mammary parenchyma. Dijkstra et al. (1997) and Hurley (2006) attributed the increase to the activities of a complex of mammogenic hormones including prolactin, oestrogen and relaxin. These hormones are responsible for proliferation of milk secreting cells resulting to increased mass of mammary tissue for galactopoiesis (Hurley, 2006). James and Osinowo (2004) and Bemji et al. (2007) reported that increase in mammary tissue was positively correlated with milk yield.

The increase in total milk yield and mammary gland traits with increased parity is expected since goats with multiples lactation cycles must have laid down more mammary structures including milk-secreting cells (alveoli) for milk production (Knight and Wilde, 1993; James, 2009).

Histological parameters including number of alveolar lobules, lactating alveoli, regressing alveoli, total alveoli, alveolar corpora amylacea, interstitial corpora amylacea, total corpora amylacea, alveolar ductular diameter, epithelial luminal diameter and epithelial height were significantly (p<0.05) influenced by bovine somatotropin dose but not by parity (p>0.05).

All histological parameters significantly (p<0.05) increased correspondingly with increased levels of bovine somatotropin doses except, regressing alveoli, alveolar corpora amylacea, interstitial corpora amylacea and total corpora amylacea where the reverse hold (tables I, II). West African Dwarf does administered bovine somatotropin have larger alveolar ductular diameter, epithelial luminal diameter and taller epithelial height (table II); more number of alveolar lobules, lactating alveoli (table I) and total alveoli and fewer alveolar corpora amylacea, interstitial corpora amylacea and total corpora amylacea (tables I, II) vis-a-vis the control.

The increased number of alveolar lobules, lactating alveoli and total alveoli and the decreased number of regressing alveoli, alveolar corpora amylacea, interstitial corpora amylacea and total corpora amylacea with increased levels of bovine somatotropin doses in WAD does treated with bovine somatotropin than in the control corroborates the findings of Baldi et al. (2002) who reported that bovine somatotropin administration to Saanen goats significantly increased the number of lactating alveoli and significantly reduced the number of regressing alveoli and corpora amylacea, both within and outside the alveolar lumen. Similar effects were

Table I. Effects of bovine somatotropin (bST) dose and parity on total milk yield, udder weight, mammary weight and volume and some mammary histological parameters in West African Dwarf goats. (Efectos de la dosis de somatotropina bovina (bST) y del número del parto sobre el rendimiento lechero total, peso de la ubre, peso y volumen de la glándula mamaria y algunos parámetros histológicos en cabras Enanas de Africa occidental).

<table>
<thead>
<tr>
<th>Parameters evaluated</th>
<th>Bovine somatotropin (bST) dose</th>
<th>p values</th>
<th>Parity</th>
<th>p values</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control (T₀) (n=3)</td>
<td>20 mg (T₁) (n=3)</td>
<td>40 mg (T₂) (n=3)</td>
<td>60 mg (T₃) (n=3)</td>
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<tr>
<td>Total milk yield (TMY, g)</td>
<td>5881.75 ± 63.17</td>
<td>8843.83 ± 85.97</td>
<td>9881.17 ± 107.83</td>
<td>10067.17 ± 120.03</td>
</tr>
<tr>
<td>Udder weight (UDW, g)</td>
<td>103.33 ± 0.98</td>
<td>112.56 ± 1.03</td>
<td>132.44 ± 1.89</td>
<td>145.11 ± 2.07</td>
</tr>
<tr>
<td>Mammary gland weight (MGW, g)</td>
<td>73.92 ± 2.17</td>
<td>85.97 ± 2.07</td>
<td>102.36 ± 2.07</td>
<td>107.36 ± 2.07</td>
</tr>
<tr>
<td>Mammary gland volume (MGV, ml)</td>
<td>63.17 ± 2.55</td>
<td>65.72 ± 2.22</td>
<td>67.61 ± 2.22</td>
<td>77.61 ± 2.22</td>
</tr>
<tr>
<td>Number of Alveolar lobules (ALN)</td>
<td>65.12 ± 0.06</td>
<td>107.83 ± 0.84</td>
<td>134.44 ± 0.84</td>
<td>182.14 ± 0.84</td>
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<tr>
<td>Lactating alveoli (LA)</td>
<td>117.02 ± 0.98</td>
<td>196.13 ± 0.86</td>
<td>218.04 ± 0.86</td>
<td>304.14 ± 0.86</td>
</tr>
<tr>
<td>Regressing alveoli (RA)</td>
<td>56.81 ± 0.33</td>
<td>12.49 ± 0.29</td>
<td>7.27 ± 0.29</td>
<td>4.01 ± 0.29</td>
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<tr>
<td>Total alveoli (TA)</td>
<td>173.83 ± 1.03</td>
<td>208.62 ± 0.89</td>
<td>225.31 ± 0.89</td>
<td>308.15 ± 0.89</td>
</tr>
</tbody>
</table>

*Means in the same row having different superscript differ significantly (p<0.05).
*Data represent means ± SE of values observed in 5 fields at magnification of ×250.
*Numbers of animals used for evaluating total milk yield (7-12 weeks of lactation) were 24. Six per treatment and 12 in each parity group.
Table II. Effects of bovine somatotropin (bST) dose and parity on some mammary histological parameters in West African Dwarf goats (Efecto de la dosis de somatotropina bovina (bST) y del número de parto sobre algunos parámetros histológicos en cabras Enanas de África Occidental).

<table>
<thead>
<tr>
<th>Parameters evaluated</th>
<th>Bovine somatotropin (bST) dose</th>
<th>p values</th>
<th>Parity</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control (T&lt;sub&gt;1&lt;/sub&gt;) (n=3)</td>
<td>20 mg (T&lt;sub&gt;1&lt;/sub&gt;) (n=3)</td>
<td>40 mg (T&lt;sub&gt;1&lt;/sub&gt;) (n=3)</td>
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<tr>
<td>Alveolar corpora amylacea (ACA)</td>
<td>7.80 ± 0.16&lt;sup&gt;c&lt;/sup&gt;</td>
<td>3.67 ± 0.14&lt;sup&gt;c&lt;/sup&gt;</td>
<td>2.81 ± 0.14&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>Interstitial corpora amylacea (ICA)</td>
<td>3.54 ± 0.13&lt;sup&gt;c&lt;/sup&gt;</td>
<td>1.49 ± 0.12&lt;sup&gt;c&lt;/sup&gt;</td>
<td>0.88 ± 0.12&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>Total corpora amylacea (TCA)</td>
<td>11.34 ± 0.25&lt;sup&gt;c&lt;/sup&gt;</td>
<td>5.16 ± 0.22&lt;sup&gt;c&lt;/sup&gt;</td>
<td>3.69 ± 0.22&lt;sup&gt;c&lt;/sup&gt;</td>
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<tr>
<td>Alveolar ductular diameter (ADD) (μm)</td>
<td>88.87 ± 1.99&lt;sup&gt;c&lt;/sup&gt;</td>
<td>101.87 ± 1.72&lt;sup&gt;c&lt;/sup&gt;</td>
<td>102.48 ± 1.72&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>Epithelial luminal diameter (ELD) (μm)</td>
<td>78.30 ± 1.97&lt;sup&gt;c&lt;/sup&gt;</td>
<td>90.27 ± 1.71&lt;sup&gt;c&lt;/sup&gt;</td>
<td>90.67 ± 1.71&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>*Epithelial height (EH) (μm)</td>
<td>9.78 ± 0.30&lt;sup&gt;c&lt;/sup&gt;</td>
<td>11.12 ± 0.26&lt;sup&gt;c&lt;/sup&gt;</td>
<td>11.98 ± 0.26&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
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</table>

Means in the same row having different superscript differ significantly (p<0.05).
*Data represent means ± SE of values observed in 5 fields at magnification of X250.

observed by Baldi et al. (2002) in Saanen goats, but lower values were observed in this study. The disparity could be due to differences in breed and doses of bovine somatotropin administered. In the present study, WAD goats, a light breed not yet selected for dairy production was used as against heavy breed Saanen goats selected for dairying. The authors used a dose of 120 mg per 2 weeks whereas a maximum dose of 60 mg per 2 weeks was used in the present study.

The larger alveolar ductular diameter and epithelial luminal diameter and taller epithelial height observed in goats treated with bovine somatotropin than in the control explains the higher secretory activity of alveolar cells. Samuelson (2007) stated that the alveoli and associated secretory tubules that comprise the secretory units of the mammary gland consist of cuboidal epithelial cells that vary in height according to their state of activity. The author further explained that each alveolar lobe undergoes a secretory cycle whereby the secretory cells increased in their height while milk is being released into the lumen and as the lumen becomes full, the secretory cells wind down in activity and decrease in height.

The effect of bovine somatotropin treatment on features of mammary gland structure in WAD does is illustrated in figures 1 and 2. T<sub>1</sub>, T<sub>2</sub>, and T<sub>3</sub> reveal lactating alveoli with various degrees of dilation of alveolar lumina with slightly thickened alveolar wall, well-differentiated and numerous cuboidal epithelial cells with abundant secretory vesicles (arrow E in T<sub>3</sub>), rounded basically displaced vesicular nuclei (arrow in T<sub>3</sub>) and increased cytoplasmic-nuclear ratio. There was relatively little or no corpora amylacea. These observations are consistent with features of mammary gland structure in WAD does indicated in Table II.

Figure 1. Representative of examples of alveolar structures in the mammary glands of West African Dwarf goats which were not treated with bST (T<sub>1</sub> control group) or belonged to a group injected with 20 mg bST (T<sub>2</sub>) or 60 mg bST (T<sub>3</sub>) (Representación de ejemplos de las estructuras alveolares de las glándulas mamarias de Cabras Enanas de África Occidental sin tratamiento (T<sub>1</sub> grupo control) o sometidas a inyecciones de 20 mg de bST (T<sub>2</sub>) o 60 mg de bST (T<sub>3</sub>)). Arrow A shows sloughed-off secretory vesicles from the alveolar wall. Arrow B shows detached secretory vesicles from the alveolar wall. Arrow C shows fewer number of secretory vesicles. Arrow D shows few number of secretory vesicles and arrow E shows numerous cuboidal epithelial cells with abundant secretory vesicles.
tions were more prominent in T1 than in T2 and T3. T2 reveals regressing alveoli showing irregularly shaped low cuboidal or flattened epithelial cells with few secretory vesicles detached and sloughed-off (arrows in T2, figure 1) and reduced cytoplasmic-nuclear ratio. There was decreased alveolar lumen with thickened interalveolar or interlobular connective tissues and presence of more corpora amylacea found within (alveolar corpora amylacea) and between alveolar cells (interstitial corpora amylacea). This is because bovine somatotropin administration to goats has been shown severely to enhance proliferation of milk secretory vesicles, maintain alveolar number and secretory activity after peak of lactation (Baldi et al., 2002; Boutinaud et al., 2003; Annen et al., 2004) with resultant effect of maintaining total mammary parenchyma weight and lactating alveoli. The reverse holds for the control where mammary apoptosis was facilitated leading to regression of the secretory epithelial cells to non-productive state (Li et al., 1999; Wilde et al., 1999).

Morphological damage of the secretory vesicles and reduction in cell number and the associated decline in milk production and tissue DNA after peak of lactation in the mammary gland of WAD does have been correlated with cell death by apoptosis (Wilde et al., 1997).

CONCLUSION

The administration of bovine somatotropin (bST) in a slow-release formulation to lactating WAD does at the onset of weeks 7, 9 and 11 of lactation increased post-slaughter udder and mammary gland weights and some histological parameters culminating to 50-71% increase in TMY of bST treated does over the control. This resulted from increased metabolic activity of the mammary cells which slow down involution, thereby allowing more secretory cells to persist over time for milk synthesis and secretion.

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