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ORIGINAL RESEARCH

The potential effect of Garlium GEM HC™ as a tick control agent in cattle

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As resistance to chemical acaricides increases amongst tick populations, investigations of natural remedies that have acaricidal or tick-repellent effects have become essential in the search for alternatives for sustainable tick control. Garlium GEM HC (Garlium), a natural concentrated garlic product, was supplemented in-feed to beef cattle to determine if it could assist in reducing the number of attached ticks on cattle. Two grams of Garlium was supplemented daily to eight Bonsmara steers over 43 days. A second group of eight steers served as controls. All animals were infested with a similar number of *Rhipicephalus appendiculatus* adults and *Rhipicephalus microplus* larvae. Subsequently, the number of attached engorged ticks and the hatchability of the tick eggs were determined. Blood samples were collected from the steers for whole blood analyses. The results were analysed using a two-sample t-test procedure. Due to large standard deviations observed, most of the tick parameters were only numerically improved. Although not significant, the number of larvae produced by female *R. appendiculatus* ticks per host was reduced by 23%. Estimated number of *R. microplus* larvae produced from ticks harvested from Garlium supplemented cattle were reduced by 42.3% ($p = 0.10$). No adverse effect was observed on haematocrits or haemoglobin concentrations. Garlium reduced segmented neutrophil count in the treatment group, suggesting that the immune system was less challenged. Although not statistically significant, the effect of reducing the number of larvae produced per host for the two tick species by approximately 20% and 40% respectively, may be of economic benefit to commercial cattle farmers.

Keywords: cattle, tick control, natural, garlic, *Rhipicephalus appendiculatus*, *Rhipicephalus microplus*

Introduction

Research institutes and commercial enterprises have focused on controlling ticks and tick-borne diseases for many years. Tick infestations on cattle adversely affect animal productivity, causing loss of blood (Little 1963) and loss of appetite (O'Kelly & Seifert 1970). This may result in a decrease in animal performance and, therefore, economic losses for the producer. Resistance of ticks to certain acaricides is a significant problem and was already reported in the 1980s when resistance to organophosphates was reported (Aguirre et al. 1986). Pyrethroid resistance in *Rhipicephalus microplus* strains was also reported (Miller et al. 1999). One of the first cases of amitraz resistance was reported in 1981 (Nolan 1981). To reduce the risk of acaricide resistance developing, it has been suggested that the alternation of acaricides or their use in combination may delay the development of resistance (Thullner et al. 2007). Using acaricides in a rotational programme appears to be effective (Jonsson et al. 2010). However, there are still too many producers that do not use acaricides responsibly, thereby increasing the risk of ticks developing resistance to certain acaricides. Continuous research and product development are needed to remain abreast of the growing development of acaricide resistance in certain tick species.

Natural alternatives to current commercial acaricides have been the focus of some research conducted as they have potential to reduce the risk of acaricide resistance. A review on natural acaricides reported that more than 200 plant species with acaricidal and tick-repellent properties are known worldwide (Quadros et al. 2020). Essential oils and plant extracts are some of the primary forms of plant-based products with repellent or acaricidal effects. Most of these plant-based products have been evaluated using a direct application approach. For example, carvacrol, derived from *Origanum vulgare,* is an essential oil with acaricidal properties. It decreased egg hatching percentage of *R. microplus* ticks when the ticks were subjected to the adult immersion test (Pereira Junior et al. 2019). *Lippia javanica* (Fever tea) aqueous leaf extract effectively reduced the number of ticks attached to cattle when the extract was sprayed onto the cattle (Madzimure et al. 2011).

Different garlic components have been evaluated to determine the effect on internal and external parasites. *Allium sativum* (garlic) and *Allium cepa* (onion) oils were primed by water, absolute ethanol, and methanol alcohols to prepare different dilutions. Direct application of the *Allium* oil solutions onto ticks killed most *Boophilus annulatus* ticks (Aboelhadid et al. 2013). The dichloromethane (DCM) extract from garlic bulbs was evaluated for its repellent effect against *Hyalomma rufipes* (Nchu et al. 2016). A tick-repelling bioassay, based on the climbing behaviour of ticks, was applied in this study. At a concentration of 1.4%, the DCM extract produced a high repellence index of 87% and 87.5% for male and female ticks, respectively. The effect of different concentrations of the methanolic extract of *Allium sativum* cloves on *R. microplus* was evaluated (Shyma et al. 2014). Larvae treated with different *A. sativum* concentrations showed significantly higher mortality than untreated tick larvae. From these publications, it is evident that garlic has acaricidal/repellent properties.

However, spraying cattle with a natural acaricide is still labour-intensive. Therefore, it would be helpful to supplement the cattle with a plant-based product, like garlic, to potentially reduce the number of ticks attached. However, as garlic is digested in the rumen, the garlic metabolites will be absorbed into the blood and distributed to different organs of the body. A few studies have been conducted to determine the efficacy of supplementing garlic-based products in-feed to ruminants.

A combination of garlic oil and cinnamaldehyde extract in-feed was supplemented at 300 mg per head per day to grazing beef cattle reducing horn fly counts of grazing heifers (Moriel et al. 2017). Another study was conducted to determine the effect of in-feed supplementation of a garlic extract product (70% mineral sulphur, and 30% sulphur compounds extracted from *Allium sativum*) on *R. microplus* that were placed bi-weekly on crossbred calves (Costa-Junior & Furlong 2011). Supplementing 20 g/h/d garlic extract over five months significantly reduced the number of engorged females by 64%, as well as the body weight of engorged females by 22% and numerically reduced the oviposition of the engorged females by 12%.

Garlium GEM HC™ (Garlium), an ADM product, is a natural product consisting of garlic powder, garlic oil and salt. Garlium was evaluated in this trial to determine the repellent effect of in-feed supplementation on the number of *Rhipicephalus appendiculatus* and *Rhipicephalus microplus* ticks attached to beef cattle as well as its effect on the reproductive success of female ticks collected from beef cattle.

Material and methods

The required ethical approvals were obtained from the University of Pretoria and the South African Department of Agriculture. Animals were cared for according to the guidelines for the Care and Use of Animals in Agriculture, Research and Teaching (1999).

The study was conducted from February to July. The experimental study was done in two stages. Stage 1 was conducted at the Experimental Farm of the University of Pretoria with 16 beef cattle. Stage 2 of the study was conducted at the Ectoparasitology laboratory at the Department of Veterinary and Tropical Diseases, Faculty of Veterinary Science, University of Pretoria.

Animals, housing and feeding

Sixteen Bonsmara bulls, aged seven months, were blocked by live weight, and allocated to two groups of eight animals each. The Control group received a feedlot starter diet with no supplementation of Garlium (CON), whereas the Garlium group received the same feedlot diet as the Control group but was supplemented with 2.0 g Garlium/animal/day (GAR). Stage 1 of the trial was divided into an adaption period of 21 days and an assessment period of 22 days. The average starting live weight of the cattle was 215 kg (± 30.8) at the onset of the trial. All animals were treated with a short-acting acaricide (Amitraz 12.5% m/v) at the beginning of the adaption period to ensure that tick infestation of the animals during the initial assessment period was low. Cattle were housed in individual pens (14 m^2) and had access to shade and *ad lib* water. Each of the beef animals in the treatment group received daily 8 kg (as is) of a feedlot diet. Diet composition (dry matter basis) was as follows: 11.17 MJ/kg; 14.3% Crude Protein and 10.4% Crude Fibre. Feed orts were recorded daily to ensure that the cattle consumed most of the feed.

To prevent the escape of ticks from the trial facility, a tick perimeter was constructed around the trial facility and filled with a deltamethrin solution.

Tick placement and collection

Laboratory-reared *Rhipicephalus appendiculatus* adults and *Rhipicephalus microplus* larvae were used in the trial. Two custom-made ear-bags (fabric tubes), one for each ear, were glued to the base of both ears of each animal using a contact adhesive. Fifty unengorged male and 50 unengorged female *R. appendiculatus* ticks were placed inside each ear-bag, whereafter the ear bags were closed. Engorged *R. appendiculatus* females were collected and counted six to eight days after the unengorged adult ticks were placed inside the ear-bags. Attached male and unengorged female *R. appendiculatus* ticks were collected and counted eight days after tick placement.

A rectangular cloth was glued onto the back of each of the cattle. A small space was left open to place an average of 3200 *R. microplus* larvae per animal between the cloth and the skin of the cattle. The opening was then closed with glue to prevent the larvae from escaping. The cloths were removed from the cattle four days after placement to allow the larvae to move around on the body. Engorged *R. microplus* females (dead and alive) were counted and collected from the floor of the enclosure 20 to 23 days after infestation. Fully engorged females were counted and collected from the animal 21 to 23 days after the placement of larvae. Unengorged females and attached males were counted 23 days post-infestation.

Live, clean, and dry engorged *R. appendiculatus* and *R. microplus* female ticks were taken to the laboratory, where they were placed into acaridariums (filled with a 40% saline solution to ensure relative humidity of approximately 85%) in a temperature-controlled room at 25 °C (± 1 °C).

Engorged ticks were counted and weighed before allowing the ticks to lay eggs for 21 days. The number of tick eggs produced per host was determined, using a water dilution method and manual counting of a representable egg sample. Thereafter the eggs were allowed to hatch (21 days from the first eggs that hatched). Larvae were then killed by freezing them for three days at -20 °C. The hatchability of eggs was then determined by counting the larvae and eggs of representative samples.

Blood samples from the jugular vein were collected on day 1 of the adaption period, the onset of tick infestation and 21 days after tick infestation to determine if Garlium supplementation had any significant effect on the main blood parameters. Twenty-gauge BD Vacutainer® needles and 4 ml EDTA BD Vacutainer® K2E tubes were used for blood collection. Whole blood analyses were conducted on the blood samples.

Statistical analyses

Data were analysed using the statistical software Genstat® (VSN International, 2017). Each animal represented an experimental unit. All parameters, except feed intake, were measured on a specific day of the trial. Animals were allocated according to their live body weight to the treatments to ensure that the average weight of the two groups was similar at the beginning of the trial. A two-sample t-test method was used to analyse the data. Predicted means were separated using Fisher's protected least significance difference test (LSD) at the 5% level. All tests declared significance at $p < 0.05$, and tendencies were accepted if $p < 0.10$.

Results and discussion

Animal production

Although, it was not the main aim of the trial to determine the effect of Garlium on performance production, the production parameters were measured and are presented in Table I.

Table I: Effect of the daily supplementation of 2 g Garlium on the production parameters of feedlot cattle

Parameter	Control	Garlium	*p*-value
Starting live weight (kg)	219.4	211.5	0.57
End live weight (kg)	279.6	273.0	0.68
Live weight gain (kg)	60.2	61.5	0.86
Aver feed intake (As is; kg/d)	8.0	7.9	0.34

There was no difference in live weight gain between the two groups over the trial period, indicating that Garlium supplementation did not affect the production performance of the cattle. The average daily live weight gain of the cattle was 1.42 kg/day for both groups and is well within the norm for beef cattle on a starter diet (Haasbroek 2014). As was expected, there was no significant difference in daily feed intake between the two groups as feed intake was restricted to a maximum of 8 kg/day, which is about 90% of unrestricted feed intake.

Tick production performance

The study aimed to determine if supplementing 2 g Garlium/animal/day will reduce the number of ticks attaching and the success rate of tick engorgement. Repellency may be assessed under field conditions, where cattle are supplemented with Garlium while grazing on pasture/veld. Natural tick infestation will occur, and the un-supplemented group can be compared to the Garlium-supplemented group over time. The benefit of such a trial is that the ticks and cattle are in their natural habitat, and ticks have the choice to attach, remain attached or detach and attempt to find another host. The challenge with a field study is that it is difficult to accurately determine the tick challenges and the number of ticks detaching from the host. Conducting a field study will indicate if the Garlium supplementation is beneficial in a commercial environment but won't necessarily provide accurate data. It will also be almost impossible to collect the engorged female ticks detached from the host to determine the egg production and hatchability of the eggs.

The alternative is to conduct a controlled study in a controlled environment, as was done in this study. The benefit of a controlled study is that data collection is more accurate, especially on the reproduction parameters of female ticks. The challenge with a controlled study is that the environment where the cattle and the ticks are kept is artificial, which will likely affect how both the ticks and the host will react. This is especially relevant for the *R. appendiculatus* used in this study. The ticks were confined to the ear (ear bag used) and did not have the opportunity of finding another host. Another potential limitation to the trial design is that only adult *R. appendiculatus* attached to the host and that both the larvae and nymphs were not exposed to the Garlium-supplemented host. There were fewer limitations regarding *R. microplus* as the larvae were placed on the host, and after a few days, the larvae were allowed to move around on the host. Therefore, all three life cycle stages were exposed to the Garlium supplementation, and ticks had the choice to detach from the host.

The effects of Garlium supplementation on *Rhipicephalus appendiculatus* are presented in Table II.

Limited research has been conducted on the effect of Garlium on tick infestation; therefore, some unpublished studies are referenced and discussed for comparative purposes. Supplementing Garlium at 2 g/animal/day had limited effect on *R. appendiculatus* (Table II). The number of engorged females was reduced by 11.9%, and males were decreased by 12.6%. As with the other *R. appendiculatus* parameters measured, the differences were not statistically significant ($p > 0.05$), most probably due to the high standard deviation per parameter within treatments.

Table II: Effect of daily supplementation of 2 g Garlium to beef cattle on average for *Rhipicephalus appendiculatus* parameters per host

Parameter	Control	Garlium	*p*-value
No engorged females	20.0 (± 10.95)	17.6 (± 9.80)	0,66
Total weight of engorged females (g)	5.13 (± 3.973)	5.05 (± 4.606)	0,97
Average weight of engorged female (g)	0.243 (± 0.1008)	0.273 (± 0.1681)	0,67
No of unengorged attached females	7.0 (± 3.81)	8.5 (± 3.34)	0,42
No of live males attached	53.6 (± 13.60)	46.9 (± 13.17)	0,33
Average no of eggs per female	2135 (± 1230)	1756 (± 1597)	0,60
Total eggs per host	44420 (± 37158)	39115 (± 46440)	0,81
Average hatchability of eggs (%)	79.5 (± 14.11)	71.6 (± 21.16)	0,40
No of larvae per host	38182 (± 35780)	30871 (± 40865)	0,71

These results are in contrast with results obtained in a commercial trial conducted in South Africa (Hagg, unpublished). In this commercial trial, 80 Bonsmara cattle were sourced for the trial and were allocated to either Control or three Garlium treatments (0.5 g, 1.5 g and 4 g Garlium/animal/day) over a trial period of 64 days. Cattle grazed on natural veld and were naturally infested with ticks. Supplementing Garlium at 1.5 g/animal/day reduced the number of attached *R. appendiculatus* by 72% ($p < 0.001$) over the 64 days compared to the un-supplemented control treatment. When comparing the commercial beef trial's results to the results of this study, it is clear that the environment may play a role in how effectively Garlium can repel ticks. Even at 1.5 g Garlium/animal/day in the commercial trial, a much better tick-repelling effect was observed than after supplementing 2 g of Garlium/animal/day in the current study. With the commercial trial, the ticks had the option of not attaching or detaching from the host and thus giving an indication of the repellent effect. With the current study, the ticks were confined to the ears, which might have limited the effect of Garlium repelling the ticks.

It was also observed in the commercial beef study (Hagg, unpublished) that supplementing 1.5 g Garlium/animal/day reduced the number of *Amblyomma hebraeum* by 39% ($p < 0.01$) over the 64-days. *A. hebraeum* is a three-host tick, similar to the life cycle of *R. appendiculatus*. Supplementing 4 g Garlium/animal/day reduced the number of attached *A. hebraeum* by 64% ($p < 0.01$) over 64 days, indicating that the supplementation level of Garlium may play a role in the efficacy of Garlium. Again, in a natural environment, the repelling effect of Garlium was more noticeable compared to the current experimental study.

The repelling effect of supplementing 1 g Garlium/springbok/day was evaluated on *Rhipicephalus evertsi evertsi* under experimental conditions using male springbok antelope and attached ear bags to the antelope (Fitte 2020). The number of engorged nymphs was reduced by 67% ($p = 0.02$) when the Garlium treatment group was compared to the Control group. In addition, the number of attached *R. evertsi evertsi* was decreased by 50% ($p = 0.01$). It should be noted that, although the *R. evertsi evertsi* were confined to the ear, the amount of Garlium/kg live weight of the host was very different (0.04 g Garlium/kg live weight for antelope vs. 0.008 g Garlium/kg live weight for the cattle in this study). This indicates that inclusion levels of Garlium are likely to play a role in the effectiveness of Garlium as a tick-repellent agent.

In this study, the hatchability percentage of the *R. appendiculatus* eggs was reduced (9.8%) in the Garlium group. However, when calculating the number of larvae produced by the female ticks per host, a 19.1% reduction in larvae was observed. Although the difference is only numerical, an almost 20% reduction in larvae produced may, over time, have a significant effect on the tick population in a commercial farming system.

Supplementing Garlium at 2 g/animal/day had a more profound effect on *Rhipicephalus microplus* than on *R. appendiculatus* (Table III). The number of engorged female ticks (dead and alive) was numerically reduced by 13.9% in the Garlium treatment. As with the parameters for *R. appendiculatus*, the differences were not statistically different, most probably due to the high standard deviation per parameter within each experimental group.

There was an observed reduction of 31.9% ($p = 0.07$) in the number of attached unengorged females when the control group was compared to the Garlium-supplemented group. This is especially important as the number of engorged females was also reduced. This may be the result of the repelling effect of Garlium since the unengorged females did not fully engorge, despite having sufficient time to engorge fully.

A significant reduction (67%, $p < 0.05$) in the number of engorged *R. microplus* females was observed in a study (Costa-Junior & Furlong 2011) where a garlic supplement was fed in-feed to beef cattle on pasture. This response is much higher than the current study's results. Possible reasons for the difference in response might be the number of larvae placed on the host (± 8 000 twice a week), whereas only ± 3 200 larvae were placed on the host once during this study. A cloth bag was not used on the body to confine larvae in a study (Costa-Junior & Furlong 2011). Thus ticks had the opportunity to avoid attaching to the host compared to the larvae confined for a few days in the current study.

Although cattle were sprayed with an aqueous garlic solution and were not supplemented with the garlic via the feed, the number of attached *Rhipicephalus* spp. (*R. decoloratus* and *R. microplus*) was reduced by 99% and 94% on day two using the 25% and 50% concentrations, respectively, compared to the negative control group (Mgocheki 2017). These results support the potential tick-repelling effect of garlic observed in this study.

Interestingly, the average weight of the live engorged females was higher in the Treatment group than the Control group, even

Table III: Effect of a daily supplementation of 2 g Garlium to beef cattle on average for *Rhipicephalus microplus* parameters per host

Parameter	Control	Garlium	*p*-value
No of engorged females (dead/alive)	234 (± 78.3)	202 (± 79.4)	0,42
No of engorged females (alive)	173 (± 64.5)	157 (± 71.7)	0,63
No of unengorged females	177 (± 66.5)	121 (± 47.4)	0,07
Total weight of engorged females (alive) (g)	46,1 (± 16.30)	42,3 (± 22.27)	0,71
Average weight of engorged female (alive) (g)	0.111 (± 0.0237)	0.129 (± 0.0193)	0,13
Average no of eggs per female	2 781 (± 269.4)	2 739 (± 137.6)	0,71
No of eggs per host	652 336 (± 219 519)	550 551 (± 215 274)	0,37
Average hatchability of eggs (%)	30.4 (± 13.00)	21.0 (± 10.81)	0,14
No of larvae per host	194528 (± 114 704)	112201 (± 62 149)	0,10

though there were fewer live engorged females in the Treatment group. This contrasts with the study (Costa-Junior & Furlong 2011), where they observed a reduction (21.6%; $p < 0.05$) in the average weight of the engorged female *R. microplus*. However, there was no difference in the number of eggs produced per female. This is supported by results reported by Costa-Junior & Furlong (2011). They observed a numerical reduction of 11.7% in the number of eggs produced per engorged female.

The hatching of tick eggs commences around the beginning of July 2020 in Southern Africa, which is the winter period. Although the temperature and humidity in the acaridariums were maintained at 25 °C and 85% humidity, the shortening of daylight length could have had a negative effect on the hatchability of the *R. microplus* eggs. Hatchability was 20–30%, which is lower than expected. Hatchability percentage of 93% (Bharkad et al. 2019) and 95% (Perinotto et al. 2012) for *R. microplus* eggs under laboratory conditions is typically expected. Average hatchability was decreased by 30.9% when the Garlium-supplemented group was compared to the control group. When considering all the parameters to calculate the number of larvae produced per host, Garlium treatment reduced the number of larvae by 42.3% ($p = 0.10$). Although this is only a tendency, a 42% reduction in larvae produced may, over time, have a significant effect on the tick population in a commercial farming enterprise. Furthermore, this reduction in tick numbers may reduce the requirement for frequent acaricide dipping of farm animals. This may lead to reduced risk of acaricide resistance of ticks on the farm.

Cattle blood analyses

Toxicity from grazing *Allium* plant species, including onion and garlic, has been associated with haemolytic anaemia in ruminants (Katsogiannou et al. 2018). A case study reported that dairy cows consumed a large number of onions (*Allium cepa*), resulting in some cows dying (Van der Kolk 2000). To determine if the supplementation of Garlium had any adverse effect on the blood, whole blood samples were collected from the cattle on different days during the study.

Supplementing Garlium to cattle at 2 g/h/d had no negative effect on the red blood cell count, haemoglobin and haematocrit concentrations. Therefore, there was no indication that supplementation of Garlium at 2 g/h/d may cause anaemia. Garlium supplementation significantly ($p = 0.02$) reduced the segmented neutrophil count in beef cattle at the end of the trial. Segmented neutrophils are part of the immune system and act as phagocytes, consuming specific pathogens. Increased levels of segmented neutrophils indicate infection or inflammation in the body (Baydar & Dabak 2014). Segmented neutrophil values were above the normal range in the Control group on the last day of the study, indicating that the animals in the Control group were possibly immunologically challenged (Bedenicki et al. 2014). Supplementing Garlium significantly reduced the segmented neutrophil counts in the treatment group, indicating that the Garlium-supplemented animals were either less challenged immunologically than the control group or the Garlium group was able to handle the immune stress better.

Conclusion

Supplementing 2 g Garlium/h/d to feedlot cattle had a limited effect on the number of ticks attaching. Although not statistically significant, Garlium treatment tended to reduce the number of *R. microplus* larvae produced per host by 42% and result in a 19% reduction in the number of *R. appendiculatus* larvae produced per host. Although the Garlium was supplemented via the feed, the garlic metabolites, in very low concentrations in the blood, have some repellent effect that could also impact the egg production of the female ticks. It is also encouraging to see that supplementing Garlium to beef cattle at 2 g/h/d had no significant adverse effects on the blood parameters.

As mentioned in the introduction, there has been an increase in acaricide resistance in tick species, such as *R. microplus,* to amitraz. The results of this study did not indicate a significant response by reducing the numbers of attached ticks by Garlium. However, when considering the other two studies done in South Africa with Garlium, a garlic-based product like Garlium can play a supplementary role in controlling tick infestations on cattle and game. An approach that could be considered is to supplement Garlium to cattle during the tick season and to only dip/spray cattle with an acaricide when the tick numbers on the cattle become too high. This will probably reduce the number of times animals need to be treated with an acaricide, thereby reducing the risk of ticks developing resistance to the acaricide. Regarding future research focus, one can try to determine the mode of action for garlic as a tick repellent agent. This may indicate on what garlic metabolites are responsible for the repellent effect, and this information can be used to improve the efficacy of a garlic-based supplement.

Conflict of interest

FM Hagg is employed by Allied Nutrition (Pty) Ltd and is responsible for the distribution of Garlium to producers. FM Hagg is also currently registered as a MSc student at the Faculty of Veterinary Science, University of Pretoria and this article reports on the research conducted as part of his MSc studies.
LJ Erasmus and WH Stoltsz declare they have no conflicts of interest that are directly or indirectly related to the research.

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Ethical approval

Prior to the commencement of the study, ethical approval was obtained from the following ethical review boards:

- DALRRD. Section 20 of the Animal Disease Act 1984 (Act 35 of 1984). Project no: REC 079 – 18. Reference no: 12/11/1/1.
- Animal Ethics Committee, Faculty of Veterinary Science, University of Pretoria. Project no: V068–18.
- Research Ethics Committee, Faculty of Veterinary Science, University of Pretoria. REC 079 – 18.

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