Chemotherapeutic Control of Trypanosomosis - A Review of Potential Drug Targets in the Parasite and Desirable Properties of Potential Drugs

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Abstract

The effective control of both human and animal African trypanosomosis has eluded mankind for decades. Vaccine production has been unsuccessful and prospects are poor apparently due to the ability of the trypanosome to change its antigenic coat and evade the host immune system. Vector control has environmental implications and has also not been successful in several areas. Drug therapy, which is the main method of control, has fallen back due to non-availability of new drugs. The existing drugs are not only old but have narrow spectra, toxic and difficult to administer. In addition, resistance to the drugs is widespread. The need to urgently develop new drugs is paramount if new outbreaks of the diseases are to be averted. Efforts to develop new drugs have been boosted by the completion of the T. brucei genome project. Application of RNA interference techniques has revealed several metabolic pathways that are unique to the parasite which can be targeted for drug development. The potential targets that have been adequately researched are reviewed here. The desirable properties of the expected drugs are also discussed.

Keywords: Trypanosomosis; Parasite; Potential Drugs

Introduction

Trypanosomosis has been with mankind for more than a century. The disease is caused by infection with various species of blood and tissue-dwelling protozoan parasites of the genus Trypanosoma that are transmitted by multiple variants of the tsetse fly (Glossina spp). It is one of the major constraints to animal health and production in sub-Saharan Africa and also has a major impact on the people’s health and livelihoods. African trypanosomes were discovered as pathogens in 1896 by David Bruce [13]. Trypanosoma brucei gambiense and T. b. rhodesiense cause fatal infection in humans especially if left untreated. In animals, Trypanosoma congolense, T. b. brucei, T. evansi, T. simiae and T. vivax cause Animal African trypanosomosis (AAT).

Anti-trypanosomal drugs are known to have featured in the pioneering work of Paul Ehrlich [30]. Ironically, development of trypanocidal drugs has not received expected priority apparently due to lack of financial incentives to develop drugs against diseases that mostly afflict poor societies [24]. The rigorous process through which new drugs must pass to be approved by drug regulatory agencies is another factor that retards the development of new drugs. Hence, Human Africa Trypanosomosis (HAT) has been classified as a neglected disease in spite of the socioeconomic effects on afflicted communities [15]. This situation has led to the search for plants with trypanocidal activities [5,24]. However, it is gladdening to know that fexinidazole has been registered in the Democratic Republic of Congo as a new all oral treatment for the treatment of stage-1 and stage-2 HAT caused by T. b. gambiense [14].

### Facts on currently used trypanocides

Although several control methods have been tried, chemotherapy remains the most viable option especially as vaccine production to the disease has not been successful because of efficient immune invasion of the parasite arising from (i) antigenic variation of the variable surface glycoprotein (VSG) coat, (ii) induction of polyclonal B cell activation, (iii) loss of B cell memory and (iv) T cell mediated immunosuppression [25]. In spite of the importance of chemotherapy in trypanosomosis control, treatment still completely depends on drugs that were developed between 30 and 80 years ago because of the sheer lack of alternatives. Suramin was introduced into HAT therapy in 1917; Pentamidine in 1939; Melarsoprol in 1947 and DFMO in 1978 [71]. Diminazene was developed in 1944 [37]. The chemical groups, dates of introduction, spectra of activity and mechanisms of action of trypanocides in current therapy are given in table 1.

<table>
<thead>
<tr>
<th>Chemical group</th>
<th>Drug</th>
<th>Year of Introduction</th>
<th>Mechanism of Action</th>
<th>Spectrum</th>
<th>Animal Species</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Arsenical</td>
<td>Melarsoprol</td>
<td>1947</td>
<td>Unknown; the disruption of the thiol-redox balance is a possible mechanism-interacting with protein sulphydryl groups and subsequently inactivating enzymes</td>
<td><em>T. gambiense</em> and <em>T. rhodesiense</em></td>
<td>Human</td>
<td>Friedheim [36]; Kuhlmann and Fleckenstein [49]</td>
</tr>
<tr>
<td></td>
<td>Melarsamine hydrochloride</td>
<td>1992</td>
<td>T. evansi, T. equiperdum, T. brucei brucei</td>
<td></td>
<td>Camel, Horse, Buffalo, Goat, Pig.</td>
<td>Giordani., et al [38]; Reynaud., et al [64]; Fairlamb [32]</td>
</tr>
<tr>
<td>Diamidine</td>
<td>Diminazene aceturate</td>
<td>1955</td>
<td>Intercalates DNA by binding to the minor groove of any organism’s DNA and disrupting translation and/or transcription</td>
<td><em>T. congolense</em>, <em>T. vivax</em>, <em>T. evansi</em>, <em>T. brucei</em>, <em>T. gambiense</em>, <em>T. rhodesiense</em></td>
<td>Cattle, Sheep, Goat, Dog, human (in chronic cases)</td>
<td>Lüscher., et al [51]; Eghianruwa., et al [28]</td>
</tr>
<tr>
<td></td>
<td>Pentamidine</td>
<td>1941</td>
<td></td>
<td><em>T. gambiense</em>, <em>T. rhodesiense</em> (variable efficacy)</td>
<td>Human</td>
<td>Seebeck and Mäser [70]</td>
</tr>
<tr>
<td>Aminophenanthridine</td>
<td>Isometamidium chloride</td>
<td>1950s</td>
<td>Not fully understood; it is hypothesized to selectively cleave kinetoplast DNA-topoisomerase complexes</td>
<td><em>T. congolense</em>, <em>T. vivax</em>. Less effective against <em>T. brucei</em>. <em>T. evansi</em>.</td>
<td>Cattle, Buffalo, Goat, Sheep, Donkey, Camel, Dog</td>
<td>Kaniisky., et al [44]; Giordani., et al [38]</td>
</tr>
<tr>
<td></td>
<td>Homidium chloride/bromide</td>
<td>1952</td>
<td>Unclear; believe to interfere with glycosomal functions, trypanothione metabolism and replication of kinetoplast minicircles.</td>
<td><em>T. congolense</em>, <em>T. vivax</em></td>
<td>Cattle, Sheep, Goat</td>
<td>Giordani., et al [38]</td>
</tr>
<tr>
<td>Aminoquinidine</td>
<td>Quinapyramine Sulphate/ Chloride</td>
<td>1950</td>
<td>Unknown; hypothesized to inhibit protein synthesis by displacing magnesium ions and polyamines from ribosomes.</td>
<td><em>T. equinum</em>, <em>T. equiperdum</em>, <em>T. congolense</em>, <em>T. evansi</em>, <em>T. vivax</em>, <em>T. brucei</em>, <em>T. simiae</em></td>
<td>Camel, Cattle, Cat, Dog, Goat, Horse, Sheep, Pig, Elephant</td>
<td>Giordani., et al [38]</td>
</tr>
<tr>
<td></td>
<td>Saramin</td>
<td>1917</td>
<td>Uncertain. Known to inhibit several enzymatic pathways</td>
<td><em>T. evansi</em>, <em>T. brucei</em></td>
<td>Human, Camel, horse</td>
<td>Seebeck and Mäser [2009]; Giordani., et al [38]</td>
</tr>
<tr>
<td></td>
<td>Alpha-Difluoroethylornithine (Efornithine)</td>
<td>1978</td>
<td>Inhibits ornithine decarboxylase; blocks synthesis polyamines (putrescine, spermidine and spermine) needed for cell growth, differentiation and replication in trypanosomes.</td>
<td><em>T. b. gambiense</em>. Leishmania, Plasmodium and Pneumocystis carinii are susceptible.</td>
<td>T. b. gambiense</td>
<td>Schofield and Kabayo [69]; Seebeck and Mäser [70]</td>
</tr>
</tbody>
</table>

Table 1: Chemical classes, ages, mechanisms of action and other properties of current trypanocides.
Apart from age, these drugs have severe side-effects, are limited in their spectrum of activities, difficult to administer and resistance is widespread for most of them [21,26]. For instance, melarsoprol, an arsenic-based drug, is administered by intravenous infusion and causes reactive encephalopathy in up to 18% [49] of treated patients. Isometamidium has been associated with tissue reaction at the site of muscular injection [11,27], a situation that causes encapsulation and reduced efficacy [28].

No single drug is effective against all the trypanosome species. Pentamidine is effective against *T. b. rhodesiense* in humans; isometamidium, diminazene and homidium are effective against *T. brucei*, *T. congolense* and *T. vivax* in animals while quinapyramine is effective against *T. simiae* in pigs. The problem is further compounded by difficulties in drug administration. Isometamidium is administered by intramuscular injection. Although significant level was reported to be absorbed from the stomach of rats [59] it was less efficacious than when injected intramuscularly [28]. The difficulty in administration can be perceived if the drug has to be constituted and injected to each animal in a herd of 1000 cattle. For drugs that must be administered by intravenous infusion, the patient must be hospitalised. These have the attendant consequences of poor compliance.

In spite of the long usage, the exact targets of these drugs in the parasite remain largely unknown as may be evident from table 1. Pentamidine, diminazene, isometamidium and ethidium are thought to target DNA [84]. These drugs likely display toxicity through disruption of host DNA metabolism. Their degree of selective toxicity may be due to two factors. First, the kinetoplast DNA may be more susceptible than host DNA to drug-induced damage because of parasite-specific features [72]. Secondly, the drugs may be selectively accumulated in the parasite [43].

**Desirable properties of new drugs**

Future goal should be to develop new trypanocides that would meet a number of criteria such as:

1. **Low cost and ready availability.**

2. **Broad spectrum of activity:** The new drug should preferably be active against all *Trypanosoma* species causing HAT and AAT. Ideally, it would be an added advantage if the new drugs are also active against other infectious agents or neoplastic diseases. This would stimulate interest of pharmaceutical companies since sleeping sickness which affects the poorest people in the world is unappealing to drug manufacturers who need to have returns on huge investments required for new drug development. Inhibitors of protein kinases [45] and N-myristoyltransferase [71] will readily satisfy this criterion as they have been found effective in cancer therapy.

3. **Ease of administration:** The new drug should be active via oral administration. For AAT, the new drug should be readily dispensed in feed or drinking water. A simple mode of administration is essential in rural conditions. Except for the newly approved fexinidazole, which is orally active [14], all other drugs available for human and animal trypanosomiasis are either administered intramuscularly e.g. pentamidine, diminazene aceturate and isometamidium chloride or intravenously e.g. suramin, melarsoprol and DFMO. The report by Brand, *et al.* [12] of orally effective N-myristoyltransferase inhibitor is a welcome development especially as it has been reported that N-myristoyltransferase could have a role in cancer therapy as well [71].

4. **Specificity and safety:** The drug should act specifically and exclusively on parasite metabolism. For instance, the proliferation of trypanosome to the procyclic stage is absent in the host physiology. Thus, inducers of trypanosome proliferation are most likely to be devoid of any activity in the host. Similarly, the absence of Trypanosome Alternative Oxidase (TAO) in the mammalian host confers high degree of specificity and thus safety to a compound like ascofuranone, a TAO inhibitor [58].

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5. **Direct delivery to target site in the parasite**: Cellular uptake of the major drugs against *Trypanosoma brucei* species is thought to occur through an adenosine transporter (P2). Drug resistance in trypanosomes has been associated with mutation in this nucleoside transporter system, thus causing reduced transport and accumulation of trypanocides in drug-resistant populations [74].

6. **New drug formulations**: Development of new drug delivery formulations are equally essential to improve parasite targeting, drug efficacy and safety. It would be a desirable property if a new drug is amenable to new formulations such as the nanoparticle delivery system. Nanoparticle based drug delivery systems possess the advantages of improved efficiency arising from ability to get the drug to the required target, reduced toxicity, prolonged drug effect, improved stability of therapeutic agents and reduction in drug dose as a result of efficient drug delivery [6]. Zelepukin., et al [86] reported that RBC-hitchhiking (delivery of particle-based theranostic agents via their transportation on the surfaces of red blood cells) can be extremely efficient for nanoparticle delivery and tumor treatment. In the case of trypanosomosis, higher efficacy of pentamidine loaded on nanoparticles of chitosan and coated by a single domain nanobody that specifically targets the surface of African trypanosomes have been demonstrated [79].

### Potential drug targets

A number of differences have been identified between the African trypanosomes and its mammalian host. Some of these include antigenic variation, energy metabolism, polyamine biosynthesis, and RNA editing [60]. These differences can be exploited as drug targets.

A potential new drug target should be a molecule that is essential to the parasite and preferably absent in the host or sufficiently different to allow selective inhibition. Knowledge of the biochemistry and completion of the genome project of *T. brucei* has revealed several potential drug targets following the identification of several proteins that are essential and unique to the parasite by large-scale gene disruption or gene silencing experiments. Drug targeting has also been advocated and employed in the search for new drugs. This involves the uptake or activation of a drug via parasite-specific pathways, as a chemotherapeutic strategy to selectively inhibit enzymes that have equally sensitive counterparts in the host [43].

The targets that have been studied to considerable extent and for which some molecules have been screened are reviewed here and also summarized in table 2.

![Table 2: Summary of potential targets and active drug molecules at each target.](image)

 cabezal de texto
Mitochondrial FoF$_1$ ATPase

Bisphosphonium compounds which are among the most promising antiprotozoal leads currently under investigation act principally on the mitochondrial FoF$_1$, ATPase in *T. brucei* [4]. These authors reported that incubation of the bisphosphonium compounds CD38 and AHI-9 with *T. brucei* rapidly inhibited the growth of the parasite and decreased ATP levels by approximately 50% within 1 hour. Other workers have also reported that benzyltriphenylphosphonium compounds display highly potent activity against trypanosomes [77]. The triphenylphosphonium (TPP) moiety of these compounds has the ability to accumulate in cell mitochondria and has been used extensively as a vehicle to deliver drugs to mitochondrial targets [76].

Differentiation to procyclic form

One widely reported hindrance to the development of vaccine against trypanosomiasis is the presence of trypanosome variant surface glycoprotein (VSG) which is the basis for antigenic variation of the parasite and escape from the host immune system. Trypanosomes have complex life cycle involving differentiation from one life-cycle stage to the next, processes that require changes in morphology, metabolism and the major surface proteins. Both the slender and stumpy forms found in mammalian blood stream are endowed with VSG but the procyclic forms to which the cell cycle arrested stumpy forms differentiate in the tsetse fly are devoid of VSG [82]. Rather, these first life-cycle stages that develop in the tsetse fly replace their VSG coat with procyclins that do not protect the parasite from lysis by host antibodies [82]. This process of differentiation from the bloodstream to the procyclic forms is specific to the parasite and can be triggered at 37°C *in vitro* [51]. Sheader, *et al.* [73] reported cell cycle arrest and rapid clearance of parasites following minimal compromise of VSG. It has been hypothesized that untimely differentiation to the procyclic form within the mammalian host would be lethal to the parasite [82]. It can be anticipated that a molecule that stimulates this differentiation would not only be a new drug candidate against Africa trypanosomes but could also be relatively safe since its mechanism of action will not be based on selective toxicity because the differentiation process is absent in the mammalian host.

Sbicego, *et al.* [68] used transgenic *T. brucei* to identify molecules capable of inducing differentiation of trypanosomes from the bloodstream forms to the procyclic forms which lack VSG and are susceptible to the host immune system. Using this method amongst others, Wenzler, *et al.* [82] screened several molecules and reported success in inducing differentiation to the procyclic forms by both the slender and stumpy forms of trypanosomes. Further studies are needed but there is no doubt that success in this area would represent new chemotherapeutic strategy against African trypanosomes.

Trypanosome alternative oxidase

The alternative respiration (AOX) is an enzyme that forms part of the electron transport chain in mitochondria of different organisms including plants. Cellular respiration by the bloodstream form of *Trypanosoma brucei brucei* reportedly depends on glycerol-3-phosphate (G3P) oxidase system which is composed of G3P dehydrogenase, ubiquinone and cyanide-insensitive ubiquinol oxidase, also known as the trypanosome alternative oxidase (TAO) [18,19]. The TAO enzyme is absent in mammals [18,55]. The absence of AOX in the mammalian host makes the *T. brucei* alternative oxidase an attractive drug target [56]. Inhibitors of alternative oxidases have been identified, the most studied of which is the antibiotic ascofuranone, a compound isolated from the phytopathogenic fungus, *Ascochyta visiae*. Nihei, *et al.* [58] following kinetic analysis of purified TAO reported that ascofuranone is a competitive inhibitor of the enzyme substrate ubiquinol. Nihei, *et al.* [58] also reported that oral (100 mg/kg) or intraperitoneal (25 mg/kg) ascofuranone in combination with 3 g/kg glycerol cleared trypanosomes from the blood of rats within 30 min of intraperitoneal or 180 minutes of oral glycerol administration. The potential of ascofuranone as a trypanocide is made more attractive by the fact that it is effective orally and also active against non-human infective trypanosomes [56].
Protein kinases

The completed genome project reveals that *T. brucei* encodes 171 eukaryotic protein kinases (ePKs) that are likely to be catalytically active, as well as 20 atypical protein kinase genes with an abundance of STE and CMGC family protein kinases [57]. The abundance of CMGC family protein kinase in trypanomatids has been associated with the need to control the complex life cycle and cell cycle of the parasites and also by the need to ensure correct replication and segregation of organelles, such as the single mitochondrion, nucleus and flagellum. Protein kinases regulate the majority of cellular pathways, especially those involved in signal transduction [34]. Several protein kinases are reportedly essential for proliferation and/or viability of parasite life-cycle stages that are clinically relevant [57]. Hammarton, *et al.* [41] reported that key cell cycle events present in higher eukaryotes are absent from trypanosomes indicating that trypanosomal protein kinases differ, at least in function from mammalian ePKs. Fundamental differences in cell cycle control between life cycle forms of *T. brucei* have also been reported [40]. These unique features of trypanosomatid cell cycle biology may be exploitable as potential drug targets for which specific inhibitors can be developed. The 4-anilinoquinazolines, canertinib and lapatinib and the pyrrolopyrimidine AEE788 reportedly killed bloodstream *T. brucei* in vitro in the low micromolar range. These compounds bind to a unique conformation of protein kinases [45].

Trypanothione reductase

Trypanothione is a form of glutathione unique to Kinetoplastida parasitic protozoa such as *Leishmania* and trypanosomes [32]. Trypanothione is essential in these parasites in the defense against oxidative stress [47]. Its absence from the mammalian host makes trypanothione-dependent enzymes suitable drug targets. Krieger, *et al.* [48] reported that trypanosomes lacking trypanothione reductase are avirulent and show increased sensitivity to oxidative stress. Studies aimed at validating trypanothione reductase as a potential drug target has resulted in the design and testing of competitive and irreversible inhibitors of the enzyme including thioridazine [50] and polyamine derivatives e.g. the naturally occurring bis (tetrahydrocinnamoyl) spermine [62].

Glycolysis

Procyclic forms of trypanosomes unlike the blood forms are known to thrive in the absence of glucose by generating ATP using amino acids through mitochondrial based pathways [78]. In contrast, glycolysis of host glucose is required for ATP production for the blood stream forms of the parasites [46]. Thus, the dependence on host glycolysis for energy can be targeted by drugs. Detailed study of the glycolytic pathway in *T. brucei* reveal compartmentalization with unique structural and kinetic features of glycolytic enzymes [61]. The first seven enzymes of the pathway which convert glucose to 3-phosphoglycerate are all located inside a trypanosome type of peroxisome, called glycosome, which is an organelle found in a few species of protozoa including the trypanosomatids [61] in contrast to the situation in other organisms where the glycolytic enzymes are cytosolic [80].

In association with glycolytic pathway, three broad targets have being proposed and they include enzymes that participate directly in glycolysis, proteins responsible for enzyme import into glycosomes and cellular components involved in the regulation of glycosome number and differentiation [20]. The glucose transport protein and enzymes that limit the rate of glycolysis in trypanosome have been identified [8]. All enzymes of the pathway have been purified, fully characterized and their 3-D structures have been elucidated as well and most have been validated as potential drug targets [3]. The enzyme triose-phosphate isomerase and glycerol-3-phosphate oxidase have been shown to be essential to parasite survival and thus seems also to be a promising target for anti-trypanosome drugs [80].

*Trypanosoma brucei* Hexose kinase, (TbHK), the first enzyme in the trypanosome glycolytic pathway has been identified as a potential target [20,46]. Studies have revealed that both TbHK1 and TbHK2 are essential to the bloodstream form of trypanosomes as cell toxicity was observed after 3 - 5 days of RNA interference (RNAi) exposure in both cases [3]. Chemical inhibitors of TbHK1 are reportedly toxic to...
the parasite [83] and have been developed as potential antiparasitic compounds. Drugs such as Lonidamine (LND, 1-(2,4-dichlorobenzyl)-1,H-indazol-3- carboxylic acid) and quercetin (QCN, 3,5,7,3,4 pentahydroxyflavone) are reported to be toxic to T. brucei through inhibition of TbHK [17]. The potential of this target is also heightened by the facts that TbHK1 shares only 30 - 33% sequence identity with mammalian hexose kinases [20] and has unusual oligomerization into hexamers [17] making specific targeting realistic. It has been reported that TbHK1 is inhibited by compounds distinct from those which inhibit the mammalian enzymes, including fatty acids [20].

**N-myristoyltransferase**

N-myristoyltransferase (NMT) catalyzes the attachment of myristic acid, a fatty acid, to many proteins [42]. N-myristoylation is needed for proper function and intracellular trafficking of these proteins. Numerous protein molecules, including several tyrosine kinases, involved in signaling cascades, oncogenesis and cellular transformation are myristoylated. Studies have shown that NMT is an attractive chemotherapeutic target against trypanosomes and other protozoan parasites such as Leishmania and Plasmodium [64]. In T. brucei, RNAi knockdown of NMT has been shown to be lethal in cell culture and to abrogate infectivity in animal models of HAT [80]. Frearson, et al. [35] reported that inhibition of T. brucei N-myristoyltransferase (TbNMT) lead to rapid killing of trypanosomes both *in vitro* and *in vivo* and cured trypanosomosis in mice. Consequently, several molecules have been developed and screened as inhibitors of TbNMT. Robinsona and Wyatt [65] reported that 19 structures of NMT from various species in complex with peptide-competitive ligands have been deposited in the Protein Data Bank and that these high-affinity inhibitors bind into the peptide substrate pocket of the enzyme and inhibit protein N-myristoylation in trypanosomes. The compounds have promising pharmaceutical properties and represent an opportunity to develop oral drugs to treat HAT [35].

The involvement of this enzyme in cancer [71], other protozoan parasites like Plasmodium, Leishmania and Candida [9,85] has been reported. The synthesis of orally active N-myristoyltransferase inhibitor has also been reported [12]. The implication of these is that an effective inhibitor could have therapeutic application in diseases other than trypanosomosis and the desirable ease of administration by oral route may be achieved.

**Protease inhibition**

Cysteine protease enzymes have attracted the attention of several researchers since the late 1990s as potential targets for new drug development against protozoan parasites including Plasmodium, Trypanosoma and Leishmania [15,23,39]. The major cysteine proteases in different species of trypanosomes have been termed rhodasin in T. b rhodesiense [15]; brucipain in T. b. brucei [81] and congopain in T. congolense [7]. These enzymes have been associated with general proteolytic activities in the parasites [7] and have been shown to be similar to mammalian cathepsin L structurally and biochemically [67]. Rhodesain, the major cysteine protease in Trypanosoma brucei rhodesiense was purified by Caffrey, et al [15]. Reversible inhibitors of the enzymes have been developed notably amongst which are thiosemicarbazones, aziridine-2,3-dicarboxylates and triazine nitrile inhibitors [29,81]. One of the tested protease inhibitors, dibenzyl aziridine-2,3-dicarboxylate displayed trypanocidal activity equipotent to the drug eflornithine [81].

**Conclusion**

The development of new drugs for treatment of both human and animal African trypanosomiasis is no doubt urgently needed even though drug development is expensive and time consuming. Attempt has been made in this review to present some of the drug targets with the highest potentials in trypanosomes. These drug targets have been revealed as a result of intensive studies to catalog the entire metabolic machinery of trypanosomes to identify functions essential and unique to the parasite for which novel inhibitors can be developed. This exercise has been aided greatly by the presence of a complete and annotated genome of T. brucei and availability of molecular biology techniques to evaluate gene function and essentiality. With the effort of public private partnerships (PPP), the renewed
focus on new drug development to trypanosomosis may in the not so distant future, change the ‘neglected disease’ status and the burden of the disease on African communities and livestock.

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Effect of Acidified Drinking Water on Gut Bacteria Community and Blood Profile of Broiler Chickens

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