CSIRO PUBLISHING

REVIEW
https://doi.org/10.1071/CP23201

CROP & PASTURE SCIENCE

# Is animal saliva a prominent factor in pasture regrowth?

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**Handling Editor:**
Brendan Cullen

**Received:** 21 July 2023
**Accepted:** 11 December 2023
**Published:** 9 January 2024

**Cite this:**
Parnell D *et al.* (2024)
*Crop & Pasture Science* **75**, CP23201.
doi:10.1071/CP23201



## ABSTRACT

Over a period spanning more than 100 years, a substantial amount of research has been undertaken to determine the impact that grazing ungulates have on grassland production systems globally, as they are the primary source of feed for these animals. Productivity of these lands, however, is highly dependent on a variety of factors such as quality and quantity of the forage, regrowth rates, and grazing rates. Expected regrowth rate of pasture, may be more influenced by animals than originally thought, as the direct effect of saliva deposition on plants on both the above and belowground biomass of plants remains relatively unclear. Though research is evident on grazing impacts on pasture, those which have utilised saliva have often found contradictory results, or do not discuss the mechanisms behind the responses in pasture observed. As such, we believe though it is a miniscule aspect of the entire grazing picture, investigating the effect of saliva in further detail may highlight gaps apparent in current research, such as what compounds are evident in saliva, and what those individual components functions are in plants, or what result may occur when applied on to plants. This review discusses what is currently known about animal saliva, the impact on pasture, and the greater practical applications of this knowledge for graziers.

**Keywords:** animal saliva, grazing, grazing interactions, multi-omics, pasture ecology, pasture production, plant growth and development, plant growth regulators, plant physiology, plant-animal interactions.

## Introduction

Grasslands across the globe are primarily used as pasture for livestock grazing; however, for effective management of nutrition and ecology, landholders must understand the determinants of intake at grazing alongside associated dynamics between animal and vegetation (Baumont *et al.* 2004). Understanding pasture productivity is highly dependent on both the quantity and quality of forage, as well the associated regrowth rates of plants. The respective growth, or regrowth rate of pasture is presumed to be influenced by the processes of grazing livestock. However, the direct effect of grazing and how it impacts pasture, both above and belowground, remains relatively unclear. As livestock are an integral part of grass-based systems, the effect of physical damage (i.e. biting) and chemical transfer (i.e. saliva deposition) by the animal to the plant, are expected to have a significant effect on various aspects of physiology through changes in plant biomass allocation and chemical elicitation of plant growth responses.

The process of grazing differs across the diversity of plant and animal species according to both the amount of pasture consumed, and the extent of interaction during grazing and mastication. Cattle differ in their interaction with pasture than sheep, and the mechanism in which livestock graze differs between the two species. For example, cattle are known to need more height in plant material as during the grazing process they use their tongues to select and remove material. In contrast, sheep can cope with lower pasture height as they graze with their teeth. This distinct difference alone in the defoliation process (i.e. ripped vs cut) can potentially lead to a varying growth responses from a grass. While most grazing studies have simulated the grazing (defoliation) processes *in situ* by the process of clipping or mowing plants, arguably however, these are not reflective of an actual grazing event in a variety of ways (Howe *et al.* 1982; McNaughton 1985). For example, studies that clip plants have had uniformity in cutting of the leaf blade, which may not be accurate due to the various ways and heights that animals defoliate a plant. The results of these types of studies have never been compared to *in situ* grazing and, as such, these studies can only be taken as an approximation of the response by a plant to grazing. Furthermore, saliva may have growth promoting effects beyond that of the concomitant effects elicited by the removal of leaf area. Studies have reported that the addition of saliva can stimulate growth in plants (Young and Schneyer 1981; Dyer *et al.* 1982; McNaughton 1985), and others have also examined the use of chemical alternatives to saliva, which have resulted in similar growth promoting responses (Reardon *et al.* 1974; Lamy *et al.* 2010; Huang *et al.* 2014; Li *et al.* 2014). However, little to no studies have explicitly determined what constituents in livestock saliva will stimulate pasture growth.

Plant growth is regulated by a range of metabolic factors; however, aboveground plant growth can be elicited by application of external plant growth regulators. Based upon our current knowledge of its biochemical composition, the growth promoting effects of livestock saliva may be realised through a combination of several mechanisms. It is presumed that biological agents with high activity may be transferred from herbivores to plants, known as 'growth factors' or 'growth regulators' during the grazing process, which in turn, has influences on plants and herbivores (Young and Schneyer 1981; Dyer *et al.* 1982; McNaughton 1985). Saliva glands are known rich sources of growth promoting chemicals for plants, such as epidermal, nerve, and transforming growth factors (Cohen 1962; Frazier *et al.* 1974). Steroid hormones have also been found alongside growth factors in saliva and has been reported to also have physiological impacts on plant growth (Dyer and Bokhari 1976; Detling *et al.* 1981, Teng *et al.* 2010; Li *et al.* 2014). Growth factors have been reported to intervene directly in cellular metabolism through promotion of differential transcription of genes (Murdoch *et al.* 1982). Vittoria and Rendina (1960) were the first to investigate chemicals being transferred to grasses during grazing and suggest that subsequently, there were positive influences on plant growth.

The impact of herbivory and its effect on plant production has been the subject of research within the past several years (Teng *et al.* 2010; Gullap *et al.* 2011) stating positive, negative, and neutral effects on grass productivity. Some grassland communities may be stimulated by grazing and result in an increase in productivity (McNaughton 1985; Frank *et al.* 2002; Schaffers 2002). Herbivory has been reported to stimulate aboveground and belowground net primary production by 21% and 35% in Yellowstone National Park (Frank *et al.* 2002), with similar values reported in Arizona (Loeser *et al.* 2004). Additionally, studies have shown that these events can also affect root development in grasses, due to the change in demand for sucrose in the root sink (Thornton and Millard 1996; Mackie-Dawson 1999; Morvan-Bertrand *et al.* 1999; Amiard *et al.* 2003). Interestingly however, saliva from other species such as bison, has been found to provide no influence on growth, yield, or activity of plants (McNaughton 1985). The influence of growth by saliva will be heavily dependent on grazing intensity, the transfer of saliva, and may also be influenced when plants are grown in limiting conditions. Therefore, further detail on what is in saliva, any discrepancies between species, and its impacts on a variety of plant species is critical.

The effect of animal saliva on pasture growth may only be a small aspect of the bigger impact of livestock grazing. However, the mechanisms of growth promotion (or inhibition) may offer useful insight and tools for understanding pasture growth. At present, the influential effects of saliva are not clear, due to the consistency of reported growth promoting effects. Due to the variability across all the studies, it is unclear what the overall mechanistic response is by plants, however, there is evidence that suggests what this response could be. Henceforth, this paper aims to review current evidence regarding the nature and magnitude of saliva application on plant growth, and the chemical composition of saliva and its variation, in order to ascertain current knowledge, and identify research objectives to characterise this important pasture-livestock interaction.

## Pasture production, terminology, and the challenges of inter-study comparisons

Grazing management can alter and impact the quality and quantity of forage available for animals. Grazing techniques and controlling stocking density can influence the above ground effects (i.e. trampling) of pasture production. The implementation of good grazing management techniques help optimise both the production of forage for livestock consumption whilst at the same time, maintaining minimum amounts of pasture residue. Pasture that is managed well helps ensure that both ground cover and root growth maintained, which helps maintain soil health and minimise soil erosion. By definition, the process of grazing in grasslands will impact on biomass accumulation and productivity. During grazing, several components can be considered in sequence that impart effects on plant physiological, biophysical, and biochemical systems. This includes defoliation eliciting wound responses, the loss of leaf surface area, changes in the ratio of plant organ sizes (e.g. root: shoot) and the deposition of saliva. All such effects impact the acquisition and allocation of plant resources including water, nutrients and photo assimilates and as such, observations regarding changes in growth rates are not uncommon. Nevertheless, evidence has suggested that application of saliva to the leaf surface elicits growth promotion in addition to these effects (Table 1) (Reardon *et al.* 1972, 1974; Dyer and Bokhari 1976; Reardon and Merrill 1978; Dyer 1980; Howe *et al.* 1982; Kato *et al.* 1993; Rooke 2003; Loeser *et al.* 2004; Teng *et al.* 2010; Gullap *et al.* 2011; Liu *et al.* 2012; Li *et al.* 2014).

**Table 1.** Summary table of papers indicating the various responses of plant species to saliva application, grazing, and manual defoliation.

| Author and year of publication | Effect (+, −, NA, none, mixed) | Plant species studied | Saliva (sampled from) or compound | Collected saliva (C), Grazed (G), manual defoliation (MD) not applicable (NA) | Lab (L) or field (F) | Effect seen/major takeaway |
|---|---|---|---|---|---|---|
| Positive effects (saliva/compounds) | | | | | | |
| Dyer and Bokhari (1976) | + | *Bouteloua gracilis* (blue grama) | Grasshoppers | C | L | • Largest effect displayed by insect grazers is increase in below ground respiration and root exudation.<br>• Grazed by grasshoppers had a faster regrowth rate than that just clipped mechanically. |
| Dyer (1980) | + | *Sorghum bicolor* (sorghum) seedlings | Mouse (submaxillary glands) | C | L | • Increase in shoot response in 3 and 6 days.<br>• Proposed that EGF or EGF like compounds provide the basis that regulate plant productivity. |
| Frank *et al.* (2002) | + (saliva effect not explicitly stated) | Mixed; *Agropyron cristatum, Festuca idahoensis, Pseudoroengaria spicata, Poa pratensis, Deschampsia caespitosa, P. compressa* | *Cervus elaphus* (elk), *Bison bison* (bison), *Antilocapra americana* (pronghorn) | G | F | • NA for saliva (not discussed)<br>• Grazing animals stimulated above aboveground and below ground production by 21% and 35%.<br>• Whole plant production increased by 32% when graziers were on the land.<br>• Root mortality increased by 22%.<br>• Predominant effect of grazers was an influence on below ground root growth (7x that of shoot growth).<br>• Aboveground productivity and grazer facilitation was positively related to consumption and grazing intensity, but weakly associated with site condition. |
| Howe *et al.* (1982) | + | *Lolium perene* (annual ryegrass) | *Sigmodon hispidus* (Hispid cotton rat) | C, G | L | • Regrowth was faster by rat grazing than mechanical clipping.<br>• Assumes that saliva contains an agent that promotes growth when coming into contact with open plant wounds.<br>• Alternatively could be due to specific manner of tissue removal which stimulates growth. |
| Huang *et al.* (2014) | + | *Leymus chinensis* | Bovine serum albumin (BSA), compound | NA | L | • BSA deposition triggers gene expression which differs to defoliation only.<br>• Established that apoptotic pathway responded to grazing stressors. |
| Kato *et al.* (1993) | + | *Vigna angularis* | Epidermal growth factor (EGF), compound | NA | L | • EGF promoted adventitious root formation in epicotyl cuttings, presumably from promotion of cell division.<br>• Most effective time periods were between 8–16 h and 16–24 h. |

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**Table 1.** (*Continued*).

| Author and year of publication | Effect (+, −, NA, none, mixed) | Plant species studied | Saliva (sampled from) or compound | Collected saliva (C), Grazed (G), manual defoliation (MD) not applicable (NA) | Lab (L) or field (F) | Effect seen/major takeaway |
|---|---|---|---|---|---|---|
| Liu *et al.* (2012) | + | *Leymus chinensis* | Sheep (mouth) | C | L | • Primarily identified that there was a link between mobilisation of carbohydrates post grazing.<br>• Animal saliva increased tiller number, buds, and biomass, but no significant effect on height.<br>• Effect also depended on intensity.<br>• Accelerated hydrolysation of fructans and accumulation of glucose and fructose. |
| Loeser *et al.* (2004) | + | Mixed species pastures including: *Pascopyrum smithii* (western wheatgrass), *Elymus elymoides* (squirreltail) and *Artemisia frigida* (prairie sagewort) | Cattle<br>Grazed and manual defoliation. | G, MD | F | • Aboveground net primary productivity (ANPP) was greater in all species when defoliated.<br>• Single year defoliated subplots had 31% higher average ANPP than non-defoliated plots, and second year defoliated subplots had 27% higher average.<br>• Clipping in plots with ongoing grazing yielded 20% higher ANPP on average than non-defoliated. |
| Reardon *et al.* (1972) | + | *Sideoats grama* | Cattle<br>Collected | C | L | • Speculated the presence of thiamine and it's influence on pasture growth.<br>• Saw an increase in plant growth response. |
| Rooke (2003) | + | *Combretum apiculatum* | Goat saliva | C | L | • Clipped shoots had enhanced growth though, unclipped shoots grew more than clips. |
| Teng *et al.* (2010) | + | *Leymus chinensis* | Sheep saliva<br>Collected | C | L | • Saliva increased leaf weight and elongation.<br>• No difference between in clipping treatments on height growth rate and aboveground biomass. |
| Positive effects (defoliation only) | | | | | | |
| Morvan-Bertrand *et al.* (1999) | NA for saliva<br>+ for defoliation | *Lolium perenne* (perennial ryegrass) | NA | NA | L | • Plants were severely defoliated, and fructan levels in leaf sheaths and elongating leaf bases strongly influenced the shoot yield during the first 2 days after defoliation.<br>• Fructans from sheaths, but fructans from immature cells may be a substrate used for growth. |
| Negative or no effects evident (saliva/compounds) | | | | | | |
| Detling *et al.* (1980) | None | *Bouteloua gracilis* (blue grama) | *Bison bison* (Bison) | C | L | • No statistical significance of foliage application on photosynthesis, root respiration, fixed C or regrowth rates.<br>• In this study, unclipped plants invariably outproduced clipped plants following defoliation after 10 days. |

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| Author and year of publication | Effect (+, −, NA, none, mixed) | Plant species studied | Saliva (sampled from) or compound | Collected saliva (C), Grazed (G), manual defoliation (MD) not applicable (NA) | Lab (L) or field (F) | Effect seen/major takeaway |
|---|---|---|---|---|---|---|
| Detling *et al.* (1981) | − | *Bouteloua gracilis* (blue grama) | Grasshoppers (*Brachystola magna*) | C | L | • Found a reduction in tillering and growth (contrasting to that of the Dyer and Bokhari's (1976) paper).<br>• Presuming the diseases/chemicals in pest saliva may have caused the reduction. |
| Johnston and Bailey (1972) | − | *Festuca scabrella* and *Festuca idahoensis* | Cattle (from rumen fistulated cow) | C | L | • After clipping plants were sprayed with freshly collected bovine saliva.<br>• No significant differences in yield of tops or roots in tiller number at any clipping height regardless of saliva. |
| McNaughton (1985) | None | *Sporbolus icolados* and *Sporbolus pyramidalis* | Thiamine, compound | NA | L | • Leaf treatment of thiamine can impact yield and metabolic balances at low applications.<br>• Defoliation rates reduced AG plant biomass to a fourth of the level produced by unclipped plants.<br>• Growth was strongly limited by defoliation.<br>• Promotion of yield and modified chemical balance of plants, reduced N concentrations.<br>• Plant species from heavily grazed grasslands were more sensitive to thiamine application. |
| Negative and no effects evident (defoliation only) | | | | | | |
| Gold and Caldwell (1990) | NA – no saliva used<br>None | *Agropyron desertorum, Artemisia tridentata* | NA – no saliva used | MD | F | • Photosynthetic rate increased following clipping regardless of defoliation pattern<br>• Increases were greater when older leaves were removed, versus younger leaves<br>• Photosynthetic competence varied with foliage age<br>• Any structural differences caused by defoliation will influence regrowth potential. |
| Harradine and Whalley (1981) | NA – for defoliation | *Aristida ramosa, Danthonia linkii* | NA – no saliva used | MD | L | • Root weight and depth were severely reduced by clipping at weekly or monthly time periods and were sensitive in roots.<br>• Defoliation led to a concentration of root mass between 0 and 10 cm (48% for *D.* linkii, 33% for *A. ramosa*) |

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**Table 1.** (*Continued*).

| Author and year of publication | Effect (+, −, NA, none, mixed) | Plant species studied | Saliva (sampled from) or compound | Collected saliva (C), Grazed (G), manual defoliation (MD) not applicable (NA) | Lab (L) or field (F) | Effect seen/major takeaway |
|---|---|---|---|---|---|---|
| Capinera and Roltsch (1980) | NA | *Tritcum aestivum* (wheat) seedlings | *Melanoplus sanguinipes* (grasshopper) | MD | L | • NA for saliva (not explicitly discussed)<br>• Focused on damage by grasshoppers to seedlings<br>• Severe defoliation by grasshoppers had lower rate of seedling regrowth than manual clipping.<br>• Chewing insects may be more difficult to simulate than generally acknowledged. |
| Hodgkinson *et al.* (1989) | NA – for defoliation | *Cenchrus ciliaris, Themeda triandra* | NA – no saliva used | MD | L | • Frequent close defoliation greatly reduced shoot production for both species.<br>• *T. triandra* was less able to withstand defoliation compared to *C. ciliaris* likely from structural and functional responses of both plants.<br>• *C. ciliaris* is likely to have evolved effective structural and functional strategies to cope with heavier grazing. |
| Jarvis and Macduff (1989) | NA – no saliva used None for defoliation | *Lolium perenne, L. multiflorum* | NA | MD | L | • Little effect of N deprivation on the growth rates of recovering defoliated shoots.<br>• Possible mechanism is the roots of N defoliated plants retains high capacity for absorption after resupply/defoliation.<br>• Stressed plants could not assimilate $NO_3^-$ or utilise it. |
| Lindgren *et al.* (2007) | NA – no saliva used – for defoliation. | *Carex bigelowii* | NA – no saliva used. | MD | L | • Clipping at different intensities with different harvest times found that the amount of soluble plant proteins is lowered in wounded plants. |
| Macduff *et al.* (2002) | NA – no saliva used None for defoliation | *Lolium perenne* | NA – no saliva used | MD | L | • The stay-green mutation in this species has no significant/adverse effect on growth of *L. perenne* during N starvation or during severe defoliation in the short to medium term. |
| Mackie-Dawson (1999) | NA – no saliva used – for defoliation | *Lolium perenne* | NA – no saliva used | MD | L | • Single defoliation event resulted in reduced tillering, biomass increment, and N uptake after 3 weeks from defoliation.<br>• Root growth was reduced by defoliation for all N impacted treatments, and in shoots that were derived from root uptake from 7 to 14 days. |

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| Author and year of publication | Effect (+, −, NA, none, mixed) | Plant species studied | Saliva (sampled from) or compound | Collected saliva (C), Grazed (G), manual defoliation (MD) not applicable (NA) | Lab (L) or field (F) | Effect seen/major takeaway |
|---|---|---|---|---|---|---|
| Stroud *et al.* (1985) | − | Western wheatgrass | NA – no saliva used | MD | L | • Simulated continuous grazing caused no major decrease in plant production.<br>• Declined however under four uniform clips. |
| Thornton and Millard (1996) | NA – no saliva used – for defoliation. | *Lolium perenne, Poa trivialis, Festuca rubra, Agrostis castellana* | NA – no saliva used | MD | L | • Clipping reduced biomass of leaf and roots when done at high intensity.<br>• Root growth decreased with increase of severity.<br>• Uptake by roots was more important in supplying N to the shoots of repeatedly defoliated grasses than remobilisation. |
| Mixed response to defoliation (with and without saliva) | | | | | | |
| Bergman (2002) | Mixed | *Salix capera* | Moose (Alces alces) | C (sedated) | L | • Increased branching on saplings.<br>• No significant effects on other growth characteristics.<br>• Consistent stimulatory effect on branching of sallow saplings, but not on growth traits. |
| Gullap *et al.* (2011) | Mixed | *Dactylis glomerata* (orchard grass)<br>*Festuca ovina* (sheep fescue) | Cattle | C | L | • Cut with saliva had greater relative height growth rate (RHGR) than controls.<br>• Saliva applied in orchardgrass increased below ground biomass in sandy based soils but decreased it for sheep fescue in the same. |
| Li *et al.* (2014) | Mixed | *Leymus chinensis* | Sheep saliva<br>Thiamine<br>Epidermal growth factor (EGF) | C | L | • No compensatory response in clipped plants.<br>• Plants in clipping with saliva had more biomass and buds than those with water or components.<br>• Clipping with salivary components had no stimulatory effects on plant growth, compared to clipping with water.<br>• Herbivore saliva had greater impacts than saliva components.<br>• No additive effect between salivary components on plant growth. |
| Reardon *et al.* (1974) | Mixed | *Sideoats grama* | Cattle and thiamine | C, G | L | • Highly significant response to height and frequency of clipping. Grazed was taller than the clipped.<br>• Plants responded to additions of thiamine but not saliva. |

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**Table 1.** (*Continued*).

| Author and year of publication | Effect (+, −, NA, none, mixed) | Plant species studied | Saliva (sampled from) or compound | Collected saliva (C), Grazed (G), manual defoliation (MD) not applicable (NA) | Lab (L) or field (F) | Effect seen/major takeaway |
|---|---|---|---|---|---|---|
| Reardon and Merrill (1978) | Mixed | *Sideoats grama* | Cattle and thiamine | C | L | • Environment can be a variable impacting response of plants to thiamine and saliva. |
| Discussions on defoliation studies (with and without saliva) | | | | | | |
| De Visser *et al.* (1997) | NA – no saliva used | *Lolium perenne* | NA – no saliva used | NA | L | • Discussed the movements of various carbohydrates in plants pre, and post defoliation. |
| Fan *et al.* (2011) | NA | Rice seedlings | Cattle Collected | C | L | • NA – this paper focused on the analysis of proteins expressed in rice seedlings after ovine saliva treatment. |
| Harris (1978) | NA – no saliva mentioned | NA – a review paper on pastures in general | NA – no saliva mentioned | NA | NA – review paper | • One of the early papers that discussed the importance of defoliation on pasture regeneration, highlighting the key influencers as frequency, intensity, and timing of defoliation. |
| Jameson (1964) | NA – no saliva used, review paper | NA – variety of species were mentioned | NA – no saliva used | NA | NA | • Summarised work that has been done on various aspects of harvesting and grazing impacts on plants.<br>• Highlighted that attention should be paid to environmental control, plant parts, and interpretation of physiology involved. |
| Kessler and Baldwin (2002) | NA – review paper | NA | Insects | NA | NA – review paper | • Review paper that highlighted the advancements of technology to understand elicited effects of insect herbivory, and responses at a molecular level.<br>• Suggested saliva may be an area of interest, and difficulty to emulate saliva. |
| Matches (1992) | NA – a review paper | NA – review paper | NA – review paper | NA | NA – review paper | • Summarises responses found of plants to grazing from animals.<br>• Grazing intensities may change morphology of the plant<br>• Animals also impact pasture composition and growth (urine/treading)<br>• Animal based defoliation early may assist in characterising plant response long term. |
| McNaughton (1979) | NA – did not look at saliva effects | *Themeda pennisteum* | Wildebeest | G | F | • NA – study did not focus on the effect of saliva explicitly, or growth response, but did focus on the energy and nutrient flow in the system. |

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**Table 1.** (*Continued*).

| Author and year of publication | Effect (+, −, NA, none, mixed) | Plant species studied | Saliva (sampled from) or compound | Collected saliva (C), Grazed (G), manual defoliation (MD) not applicable (NA) | Lab (L) or field (F) | Effect seen/major takeaway |
|---|---|---|---|---|---|---|
| Miles and Lloyd (1967) | NA – study did not look at saliva and growth rates | Sunflower seedlings | Insects | NA | L | • NA for saliva explicitly, but the study identified plant damaged caused by saliva of these insects.<br>• Saliva of aphids converted tryptophan to B-indolyl acetic acid (IAA) and contains various amounts of this plant hormone.<br>• Results indicated that synthesis of IAA occurs naturally during the salivation of these insects. |
| Parsons *et al.* (1983) | NA – study did not look at saliva and growth rates | *Lolium perenne* (perennial ryegrass) | Sheep | G | F | • Hard grazed had highly efficient photosynthetic activity (77% net activity).<br>• Photosynthesis was substantially less than leniently/lightly grazed sward. |

+, positive effect; −, negative effect; NA, not applicable.

Deposition of saliva onto grasses during grazing occurs as animals lick forage to direct biomass into their mouths, or the mouth come into contact with grass; both of which leads to the transfer of chemicals to the un-grazed plant tissue (McNaughton 1985; Liu *et al.* 2012; Huang *et al.* 2014). It is generally presumed that saliva deposition is a cue for plants to stimulate growth and leading to the initiation of the compensatory growth response (McNaughton 1979). While it generally hypothesised that herbivory (grazing by livestock) has positive influences on pasture growth, increasing fitness and competition (Vittoria and Rendina 1960; Dyer and Bokhari 1976; McNaughton 1979; Detling *et al.* 1980, 1981; McNaughton 1985; Matches 1992; Bergman 2002), a number of studies have observed that also suggests either negative, or no impacts at all to plants (Johnston and Bailey 1972; Reardon *et al.* 1974; Capinera and Roltsch 1980; Detling *et al.* 1981). As such, despite the paucity of research regarding the effect of saliva on plant growth, it is generally believed that under certain conditions growth promotion is likely to occur. Its therefore prudent to investigate potential mechanistic explanations of how this might occur.

## Growth promotion

The terminology describing the process of plant biomass removal during the grazing process has not been consistently applied among previous studies and has implications for interpreting any *in situ* and *ex situ* treatment effects. For example, there are papers that have discussed animals ‘defoliating’ a plant, whilst others mention ‘grazing’, and few too have discussed ‘defoliation’ as a form of mechanical/manual clipping only. As such, in this paper, we use the term ‘grazing’ as the process in which livestock licks, bites, rips, and swallow pasture and ‘defoliation’ as the process by which pasture is mechanically cut to emulate the process of grazing. In either method, leaves are either completely or partially removed. This in turn has impacts on the photosynthetic activity, secondary metabolite activity, and carbohydrate relocation of the plant (Gold and Caldwell 1990; De Visser *et al.* 1997; Macduff *et al.* 2002). Though responses to grazing/herbivory and defoliation/clipping events may have similar results, it is not feasible to substitute one for the other when discussing the effect of animals grazing on pasture (White 1973; Capinera and Roltsch 1980). Where the effect of mechanical clipping/defoliation is used as a substitute for actual grazing and the subsequent research has focused on; e.g. plant photosynthetic capacity, root growth or nutrient uptake, the vast majority of cases does so without the added influence of saliva (Clement *et al.* 1978; Parsons *et al.* 1983; Jarvis and Macduff 1989; Christopher *et al.* 2004) (Table 1). Additionally, while studies have investigated the role of defoliation only (Heady 1961; Jameson 1964;

Harris 1978; Stroud *et al.* 1985; Lindgren *et al.* 2007; Deutsch *et al.* 2010; Wang *et al.* 2020, 2022; Koptur *et al.* 2023), these studies ignore the potential impact that saliva has on stimulating plant growth (Reardon *et al.* 1972; Detling *et al.* 1980; McNaughton 1985; Teng *et al.* 2010; Liu *et al.* 2012) (Table 1).

There are a plethora of plant responses as a result of mechanical clipping and grazing which are not clearly understood, as there are a range of factors in play that may alter the response. For example, the presence of an animal and their influence on nutrient cycling in the ecosystem is one of those factors. Studies have also previously suggested that the vast majority of nitrogen, phosphorous and potassium in forage consumed by graziers is returned to the pasture in the form of faeces or urine. However, in greenhouse studies where plants are defoliated, this material is typically removed from the system altogether (Sears 1951; Peterson *et al.* 1956). Another example is the height at which plants are clipped, compared to what is observed by grazing livestock such as clipping occurring at a random height in an attempt to emulate livestock bite patterns (Jameson 1964) or has been performed at levels far more severe than what occurs during grazing events (Heady 1961).

In addition to variability in application of defoliation (vs grazing) treatments, plants have evolved a variety of adaptive mechanisms, which makes them more resilient to the grazing/defoliation process and as a result, plants differ in their responses to grazing by herbivores (Harradine and Whalley 1981; Hodgkinson *et al.* 1989; Gullap *et al.* 2011). Furthermore, environmental conditions such as plant growth stage, soil, nutrients, weather, and climate play a large factor in forage growth (Reardon and Merrill 1978; Buxton and Mertens 1995; Loeser *et al.* 2004; Schacht and Volesky 2010; Gullap *et al.* 2011), also influence growth responses after a defoliation/grazing event. Finally, plants are rarely grazed only a single time so the frequency, intensity, and timing of defoliation or grazing events will also influence a plant response to grazing/defoliation events (Harris 1978; Gullap *et al.* 2011).

Additionally, previous research has reported on saliva and defoliation events on *Bouteloua curtipendula* (Reardon *et al.* 1974), *Sporbolus ioclados* and *Sporbolus pyramidalis* (McNaughton 1985), *Agropyron smithii* (Stroud *et al.* 1985), *Salix caprea* (Bergman 2002), *Carex bigelowii* (Lindgren *et al.* 2007), *Leymus chinensis* (Liu *et al.* 2012). In an Australian context, few plant species have been investigated with regard to their response to saliva and defoliation events. Notably, predominant native Australian pasture species such as *Themeda triandra* (kangaroo grass), have not yet been investigated. As Australian native grasses often respond poorly to grazing due to their ability to easily be overgrazed and poor responses to fertilisation treatment (Garden *et al.* 2001; Mokany *et al.* 2006; Nie and Zollinger 2012; Mavromihalis *et al.* 2013), and the generally poor adaptation of grasses to grazing events in general, it is critical to identify how the intricate process of grazing impacts the regrowth of these species, and identify new insights on how grazing impacts not only native grasses, but also on a range of introduced grasses that are a critically important component of much of Australia's extensive livestock production systems. Therefore, to manage pasture in the most appropriate manner to maximise productivity, it is necessary to understand, and quantify the response of grass to grazing activities, as distinct from grass defoliated using artificial methods, often explored *in situ*.

## Chemical constituents of saliva and its physiochemical properties

Saliva is comprised of a series of compounds that assists in masticating and swallowing and is secreted in large quantities and at various rates during resting, eating, and rumination (Kay 1960; Bailey and Balch 1961). Saliva in ruminants contributes water and salts to the rumen and has a pH of approximately 8.4 (Bailey and Balch 1961). Previous research has found that the submaxillary glands secrete saliva during grazing (480 mL $h^{-1}$ by cattle; (Ellenberger and Hofmeister 1887), whilst the parotid glands secrete continuously but at an increased rate during mastication of food (Bailey 1961). Saliva is produced in glands that are present all over the mouth; however, the major glands function through connective ducts to the parotid and mandibular glands, which are innervated parasympathetically (Colin 1886; Bailey 1961; Bailey and Balch 1961). Of note is the type of secretions these two major glands produce in livestock: (1) the parotid salivary gland, which is known to produce serous based secretions (water based, rich in proteins); and (2) the mandibular glands that produce a meocrine solution (a water and mucous mixed solution) comprised of mucopolysaccharides and glycoproteins (Nonaka and Wong 2022). The mandibular gland is the largest gland found in livestock (Dehghani *et al.* 1994; Dehghani *et al.* 2000) and produces more saliva than the parotid gland. At the mandibular level, there are two primary glands that secrete saliva within the oral cavity: (1) the submandibular gland; and (2) the sublingual gland (Dehghani *et al.* 1994; Dehghani *et al.* 2000; Ellis 2012). The submandibular gland, the larger gland located near the mandible, releases saliva into the mouth through the ducts, whilst the sublingual glands are located beneath the tongue, which releases saliva produced into the floor of the mouth (Ellis 2012). Buccal salivary glands are minor salivary glands, located along the inner side of cheek within the mouth (Ellis 2012). Essentially, saliva is a result of continuous secretion of parotid, mandibular, sublingual, and buccal salivary glands, and rate of secretion can depend on factors such as gland type, stimuli, individual animal, diet, and activity (Kay 1960).

Whilst little is known about specific properties of livestock saliva that may induce a growth response in plants, prior exploratory analysis of biomarkers within saliva however has observed common constituents such as water, various proteins, salts, electrolytes, and antimicrobial agents (McDougall 1948; Chauncey *et al.* 1954, 1963; Chauncey 1955; Kay 1960; Young and Schneyer 1981; Turunen *et al.* 2020). Authors have speculated presence of notable compounds such as thiamine (Bonner and Greene 1939; Reardon *et al.* 1972), and bovine serum albumin (BSA) (Vittoria and Rendina 1960; Reardon *et al.* 1974), which may in turn be responsible for positive growth responses post defoliation. Despite some qualitative understanding of the constituents of livestock biofluids (i.e. urine, ruminal fluid), no descriptive, 'omic'-based approach to chemical characterisation of livestock saliva has been conducted to date. Proteomics, metabolomics, and ionomics are studies which can be undertaken to identify the presence of proteins, various metabolites (i.e. peptides, carbohydrates, lipids) and ions, respectively. These studies allow for a deeper understanding of novel molecules, various concentration ranges and presence with biological samples. Little is known about the scope of chemical diversity nor the quantitative range chemical constituents, both of which may contain valuable information regarding the mechanisms of growth promotion. However, a summary of what compounds are known to be present in saliva (regardless of species of animal that they have been obtained from), those which have been speculated to be present in livestock, and the role they may have on influencing grass growth and productivity following a grazing event, would be beneficial. We present below chemical candidates from existing literature, categorised in to compound classes.

## Metabolites

Currently within the literature, the metabolite proposed as an active component within saliva for plant growth promotion is thiamine. Thiamine is an essential micronutrient which aids in the growth, development, and function of cells (Addicott 1941). It is a water-soluble vitamin that has a N containing ring based in the centre and is essential in a phosphorylated form for metabolic reactions associated with the breakdown of carbohydrates and amino acids. Thiamine has been classified as a growth factor, often produced in the shoot and is necessary for root and shoot growth in plants (Bonner 1937, 1940; Robbins and Bartley 1937; Bonner and Greene 1939; Addicott 1941; Clark 1942; Vittoria and Rendina 1960; Reardon *et al.* 1972, 1974; McNaughton 1985). Thiamine is active in root nodule and mycorrhizal symbioses in which it has a profound effect on nitrogen and phosphorous uptake and thus on plant growth and development (Fitzpatrick and Chapman 2020). Previous publications have shown that animal saliva contained thiamine at concentrations that would stimulate a growth response in grasses (Bonner and Greene 1939; Reardon *et al.* 1972). However, in spite of this, there has been little to no evidence presented in the literature as to why it is considered a stimulator of plant growth following grazing, as the simple presence of thiamine in saliva can in no way be used to conclusively show that it does fact stimulate plant growth. As such its presence in a saliva substitute or an artificial saliva may or may not influence plant growth.

## Proteins and their derivatives

Several proteins and their derivatives in saliva have been postulated as having a positive growth promoting effect on plants however, no candidates are explicitly correlated with growth promotion and no study has mechanistically proven such an effect. Understanding their chemical composition, functional groups and in the case of protein activity; substrate affinity and enzyme kinetics, may offer some insight into the functional activity of these chemical compounds. BSA is a protein derived from bovines (cattle) and are primarily responsible for providing colloid osmotic-pressure within capillaries, transporting fatty acids, minerals, and hormones (Peters 1996; Chen *et al.* 2021). In addition, BSAs also function as an anti-oxidant and -coagulant (Peters 1996). BSA has been used due to it being a common blood protein found in livestock, often a carrier for steroids, fatty acids, and thyroid hormones, and has been studied in plant research for over decades (Vittoria and Rendina 1960; Reardon *et al.* 1974). It has been suggested previously that BSA is the protein that interacts in plants, with signs of cell death and various activities (i.e. stress, and transport) being evident when applied (Lamy *et al.* 2010; Huang *et al.* 2014). Additionally, epidermal growth factors (EGF) are known to enhance growth, promote adventitious root formation, and cell division of epicotyl cuttings (Kato *et al.* 1993), and have been speculated to be the compounds responsible for how herbivores may aide in regulating plant productivity (Dyer 1980).

The enzyme amylase is a major digestive enzyme (Daja and Treska 2015) and is also present in saliva, which is the chemical elicitor that begins the digestion process as it is responsible for breaking starch into maltose and dextrin (Fried *et al.* 1987). In animals, the primary form of amylase is α-amylase and is produced in the salivary glands. In contrast, plants predominantly have beta-amylase, though both α-, and γ-amylase classes are also present (Azzopardi *et al.* 2016). However, as in ruminants, amylase in plants breaks down starch into sugar to provide energy for the plant during early germination stages. The conversion of large molecular weight compounds to low molecular weight compounds by amylase during starch metabolism increases the osmotic potential of the cellular solution and may impart a growth response through increased osmotic pressure, leading to cell expansion, as well as providing increases in the availability of substrates for metabolism. Lingual lipase is another enzyme present in saliva that is a part of the digestive process (Hamosh and Scow 1973). In animals, lingual lipase catalysis the digestion of lipids initially in the mouth, which continues to occur in the stomach (Hamosh and Scow 1973). Lipases are also present in plants; however, they are primarily found in tissues of growing seedlings (Pahoja and Sethar 2002) where they catalyse they hydrolyse triacylglycerols to fatty acids to be converted to sugars that support the growth of seeds during the process of germination (Lin *et al.* 1987).

Glycoproteins are proteins that are integral membrane proteins and play a role in cell-to-cell interactions. Glycoproteins are predominantly N-linked and O-linked and are often found in the body as mucins (Gamblin *et al.* 2009). Glycoproteins have a variety of functions including acting as a structural molecule within collagen, a lubricant/protective agent in mucins, an immunologic molecule in immunoglobulins, and hormones such as thyroid stimulating hormone (Murray *et al.* 2006; Maverakis *et al.* 2015). In plants, glycoproteins are present in plant cell walls and assist in adaptation of a plant to its environment (Selvendran and O'Neill 1982; Josè-Estanyol and Puigdomènech 2000). Mucin is a glycosylated protein, and a macromolecular component of the 'mucus' component of saliva (Voynow and Fischer 2006). Mucus is a mixture of water and a diverse range of 'defensive proteins' that are largely comprised of glycans of both the N-linked and O-linked type (Voynow and Fischer 2006; Dolan and Hansson 2023). Mucin in livestock originates from secretions in submaxillary glands, and its function is to aid in digestion so food can easily travel through the digestive tract (Proust *et al.* 1984). Whilst there is no obvious mechanism by which mucin can stimulate plant growth, saliva is a crucial line of defence against microbial species due to its richness in antimicrobial compounds. Antimicrobial agents in this instance are natural substances that can kill or inhibit the growth of microorganisms such as bacteria, fungi, or algae (Burnett-Boothroyd and McCarthy 2011). Secretory immunoglobulin A (SlgA) is a known antimicrobial agent produced in large amounts in animal saliva and stimulates immune protection and preservation of immune homeostasis (Mach and Pahud 1971; Myer *et al.* 2015; Fouhse *et al.* 2017). Other antimicrobial proteins evident in saliva include lysozyme (Salton 1957), lactoperoxidase (Singh *et al.* 2012), and lactoferrin (Sánchez *et al.* 1992). Of the aforementioned proteins, only two are expressed in plants, lysozyme and peroxidase, which serve the same antimicrobial function in plants. Peroxidases function in many of the physiological processes in plants, such as biosynthesising lignin, and similarly to animals, acting as a defensive agent against pathogens and wounding (Fujiyama *et al.* 1995; Vicuna 2005). In plants, physiologically, lytic vacuoles achieve a similar outcome to lysosomes in animals, as these vacuoles degrade cellular materials (Hara-Nishimura and Hatsugai 2011) whilst lysosomes digest waste in a cell (Salton 1957). Mucopolysaccharides, or glycosaminoglycans, are comprised of repeating two-sugar units (disaccharides) and are found in bacteria, vertebrates, and invertebrates (DeAngelis 2002; Esko *et al.* 2009). The former is often found in mucus and fluid in joints. Mucopolysaccharides also vary in their mass, structure, and sulfation, and can be further subdivided depending on their main structures (Sasisekharan *et al.* 2006). Their function in animals ultimately depends on which of the four classes the mucopolysaccharide belongs to; for example, cellular wound repair, brain development, pathogen infection, and functions in skin, vessels, and heart valves (Funderburgh 2000; Trowbridge and Gallo 2002; Sugahara *et al.* 2003; Tortora 2013). The mechanism in which glycosaminoglycans function on plant growth, however, has not been described (Yamada *et al.* 2011).

### Ions

Electrolytes include most soluble salts, acids, and bases, and may be either positive or negative ions or minerals that help the body maintain fluid balance and a constant pH (Enderby and Neilson 1981; Ruckebusch *et al.* 1991). The primary ions in electrolytical physiology are sodium, potassium, calcium, magnesium, chloride, hydrogen phosphate, and hydrogen carbonate (Enderby and Neilson 1981). Sodium in particular, is the main electrolyte found in the body and is involved in blood pressure control (Enderby and Neilson 1981). Though little research has been undertaken on the extent of plant growth promoting ions in livestock saliva, there are studies on electrolytes (i.e. sodium, chloride, and potassium) in livestock for other areas of research such as patterns during oestrus and pregnancy (Devi *et al.* 2016; Mojsym *et al.* 2022), in calves (Kumar and Singh 1981), and in long term research on electrolyte changes (Grimm *et al.* 2021). Whilst the physiochemical interactions of free ions are likely limited to their influence over osmotic potential (and increasing cellular turgor pressure), induction of signal cascades by increase in ion abundance (such as potassium which affects plant hexokinase activity) is observed in both plant and animal cells (Alberts *et al.* 2002).

## Species specific interactions and sampling protocols limit extrapolation of results

Despite the physiochemical properties of saliva providing some support for the notion that it imparts or stimulates a growth response on plants, our ability to make broad scale predictions of growth promotion is limited by several factors. In part this is due to the fact only a handful of animal species have thus far been investigated. When saliva has been included in studies such as metabolomics or proteomics, it has been predominantly studied in species such as hogs, grasshoppers, goats, rabbits, rats, dogs, and humans (Palmer 1916; Hines and McCance 1953; Chauncey *et al.* 1954, 1957, 1958, 1963; Chauncey 1955; McGeachin and Gleason 1956; Cohen 1962; Dyer and Bokhari 1976; Rooke 2003; Turunen *et al.* 2020) (Table 1) but not in livestock, or was included in studies when analytic capabilities were limited (Reid and Huffman 1949). Recent research has focused on a variety of other biofluids from cattle, though saliva has seldom been the focus of these studies when included. For example, metabolomic investigations performed more recently in cattle included analysis on milk, ruminal fluid, serum, urine, and faeces (Kim *et al.* 2021; Zhu *et al.* 2021). The chemical constituents of saliva in sheep however have been characterised extensively in sheep (Chauncey *et al.* 1963; Lamy and Mau 2012; Palma-Hidalgo *et al.* 2023) and work by Lamy and Mau (2012), and Palma-Hidalgo *et al.* (2023) focused on proteomics and metabolomics-based research, respectively. A literature review by Goldansaz *et al.* (2017) surveyed metabolomic studies undertaken between 1930 and 2015 did not include saliva as a biofluid within their search parameters. However, this could be due to protein analysis of animal biofluids being a new area of research for factors such as diseases and hormones (Lamy and Mau 2012). Moreover, when bovine saliva has been highlighted in 'omics'-based research, there is often little interest expressed in its role as elicitor of growth and the responses of a plant to saliva application, and studies instead focus on what has been expressed by the plant post application; i.e. protein expression (Fan *et al.* 2011).

These studies highlight another factor affecting our ability to infer firm conclusions; that is, the role that saliva collection may play on the data collected and particularly regarding cross study comparisons. Of the studies investigating sheep saliva, research by Lamy *et al.* (2010) had saliva sampled from animals under anaesthetic, with catheters sampling directly from the parotid gland. In contrast, research by Palma-Hidalgo *et al.* (2023) had saliva sampled by swabbing the mouth of sheep and may provide a more accurate representation of the constituents of saliva in the mouths of animals at the time of grazing. Research performed by Reardon *et al.* (1972) collected saliva from cattle from the mouth, whilst that of Johnston and Bailey (1972) collected saliva directly from the rumen. These variations in collection technique undoubtedly have implications for interpretation. For example, saliva from the rumen may contain detrimental contaminants such as bacterial enzymes (Reardon *et al.* 1974). Though the former three studies were not focused on an 'omics approach to understanding saliva impacts, it is still critical to highlight the importance of 'omic characterisations of saliva based upon standardised protocols, including that of saliva collection.

Previous studies have also attempted to use 'synthetic' saliva as a substitute, and was a concept initially proposed by Jameson (1964). However, results of studies that used a synthetic version have often suggested that the growth response of plants do not elicit the same response compared to the use of real saliva (Reardon *et al.* 1974; McNaughton 1985; Lamy *et al.* 2010; Huang *et al.* 2014; Li *et al.* 2014). Animal saliva used in contrast, has been found to have a larger impact on plant growth than any synthetic substrate used, further contributing to the complexity of understanding the impact that individual substrates may have (McNaughton 1985; Li *et al.* 2014). Huang *et al.* (2014) proposed that herbivore saliva is 'unstable' possibly due to the presence of bacteria, which further reiterates the need for characterisation of livestock saliva, due to the potential interactive effects between the different components within saliva. This interactive effect further highlights the overall complexity of salivary composition. The various protein and growth factors present in saliva, regardless on the level of individual presence, may not be encapsulated in a synthetic mixture and therefore, not accurately resemble saliva itself (Cohen 1962; Miles and Lloyd 1967; Kessler and Baldwin 2002). Furthermore, there are likely to be large costs of making a synthetic solution due to the mixture of proteins and growth factors evident.

Applications of this knowledge include the ability to design experimental procedures, which can identify growth promotion as separate to other factors such as damage and wound responses. Along with a multi-omic approach to investigate what is in saliva, it is suggested that studies should be performed to identify what the potential contributors to plant growth are by testing all salivary components individually, to rule out if this effect is likely due to the addition of growth promoters, proteins, and nutrients, or if it likely a wound response by the plant. Additionally, research should be undertaken to determine if a salivary response differs in livestock species (i.e. cattle or sheep) and breeds within the species (e.g. a Jersey or Angus in cattle). Research should also be devoted to increase the range of species of pasture studied both *in situ*, and in field, so an extensive library can be created on the various growth effects that saliva has. By doing so, we will develop a much clearer understanding of how grazing impacts pasture production, including stimulatory responses, in order to understand the extent that plant-animal interactions can be better utilised to maximise the sustainable productivity of grazed systems.

## Conclusion

Despite the extensive amount of existing research, many questions remain regarding the impact that saliva has on pasture production. These range from understanding the contradictions present in the existing literature on the effects that saliva has, all the way through to determining what is present in ruminant saliva both qualitatively and quantitatively. Through investigation of existing literature, out of the 21 defoliation studies found to have utilised saliva and/or various constituents presumed to be in saliva, only 11 studies identified a positive influence of saliva on plant growth, and five studies identified a mixed effect. Evidently, more research is required to understand why certain constituents within livestock saliva might cause growth promotion (or inhibition) post-grazing. More recent analytic techniques such as multi-omic approaches to determine what is in livestock saliva show great promise here. Studies such as those would allow a deeper understanding of the constituents within saliva, and so too, which compound may impact growth responses by plants. With the ability to isolate the active constituents that promote growth in plants, a chemical tool may be identifiable, and utilised to enhance the resilience of pasture production systems. Although the likely impact of saliva overall being of a minute scale when at a paddock level, the influence of saliva on individual plant growth is likely to be a significant contributor to overall pasture production.

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**Data availability.** Data sharing is not applicable as no new data were generated or analysed during this study.

**Conflicts of interest.** The authors declare that they have no conflicts of interest.

**Declaration of funding.** The authors acknowledge the Coolringdon Research Trust that provided a scholarship to the corresponding author (DP).

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