

Harnessing of Proteomic and Molecular Analysis of Bali Cattle Saliva: Another Side to Study the Potential of Bali Cattle (*Bos javanicus*) as a Native Indonesian Cattle

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Harnessing of Proteomic and Molecular Analysis of Bali Cattle Saliva: Another Side to Study the Potential of Bali Cattle (*Bos javanicus*) as a Native Indonesian Cattle

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ABSTRACT

Bali cattle are native to Indonesia, with some superior properties such as high fertility, remarkable resistance to heat stress as well as to most diseases that lead Bali cattle are suitable for tropical regions such as Indonesia and may be for other tropical countries as well. Up to now studies have been focused on their main purpose as small beef cattle, in fact Bali cattle saliva has also shown potential as a source of biological material. This book chapter discusses the studies that have been carried out in the last five years regarding saliva of Bali cattle from proteomic and molecular aspects. From this discussion it is hoped that further thoughts will be born to explore the Bali cattle from other aspects. Hopefully the information is also useful to explore the benefits of saliva in other native animals besides Bali cattle.

Keywords: Bali cattle; Bos sondaicus/javanicus; saliva; proteomic; biological material.

1. BALI CATTLE, ITS ORIGINS AND ROLE IN INDONESIAN SOCIETY

Bali cattle are native to Indonesia and are derived from domesticated banteng, *Bos javanicus*, *Bos banteng* [1,2]. The striking features of Bali cattle are that the females have bright red-copper to dark skin, while the males are blackish brown in color. Both in males and females, there is a white, oval-shaped skin marking on the buttocks extending to the inner thighs, and also on the lower leg to the knee (tibia/fibula) so as if they are wearing white socks (Fig. 1). If observed in more detail, a black line is clearly visible along the backbone from head to the base of the tail of both male and female Bali cattle.

In 2019, according to Director General of Animal Husbandry and Animal Health, the Ministry of Agriculture of Indonesia, the population of Bali cattle was around 5.5 million or approximately 33 per cent of the beef cattle in Indonesia [3].

Compared to those from other breeds – although they are fed on poor-quality fodder – Bali cattle have several advantages, such as high fertility, remarkable resistance to heat stress as well as to most diseases [4]. The superior properties of Bali cattle are thus very suitable for tropical regions such as Indonesia and may be for other tropical countries as well.

In rural areas, in the beginning, when agricultural mechanization was still expensive, many Bali cattle were used to plow the rice fields during the rice planting season. At present Bali cattle are more

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directed to produce meat even though their body size is relatively small to beef cattle in general, which are around 250-350 kg, despite the fact that some also reach 600-800 kg in weight [5,6].



Fig. 1. Typical features of Bali cattle, a group of cow with a bull with dark skin (Courtesy Prof. Sukardono, Faculty of Animal Science, Mataram University)

The superiorities of the Bali cattle have made researchers interested in conducting a deeper study of the Bali cattle, especially those related to its physiological status.

Regarding the study of the physiological status of Bali cattle, their saliva is very suitable to be used as a biological material, rather than using blood, or milk. The reasons are that the majority of farmers are not happy for their livestock to have their blood drawn, although the purpose is for controlling the health conditions of their livestock. Furthermore, Bali cattle are not a type of dairy cows, the use of milk as biological material is therefore impracticable as well. For these reasons, saliva is therefore ideal to be used as a diagnostic medium for non-invasive assessment [7,8].

This article discusses the importance of understanding about bovine or ruminant saliva from the biological, proteomic, and genomic aspects. From here in it can be expected that new breakthroughs will emerge to study ruminant biological status by means of techniques that as far as possible from invasive methods that make not only the animals, but also farmers become uncomfortable.

2. THE POTENTIAL OF BALI CATTLE SALIVA AS A SOURCE OF BIOACTIVE COMPOUNDS AND AS A MEDIUM FOR NON-INVASIVE EXAMINATION

Saliva is a complex and unique biological fluid, secreted into the oral cavity by glands (parotid, submandibular, and sublingual glands) as presented in Fig. 2. As a body fluid with a leading position in the digestive system, saliva has become a special fluid to prevent the entry of pathogens into the body, especially the digestive system (reviewed by Depamede et al. [9]). Cattle can produce saliva up to 220 L or more per day [10], and their saliva also plays a role in providing sufficient fluid for rumen fermentation and acting as a buffering agent.

Regarding the biological or physiological function of cattle saliva, especially for Bali cattle, we have conducted a preliminary study that examined a bioactive compound in the saliva by conducting a partial isolation using aqueous mixture of polyethylene glycol (PEG) with sodium sulfate on a compound with a molecular weight of around 14.2 kDa (Fig. 3).

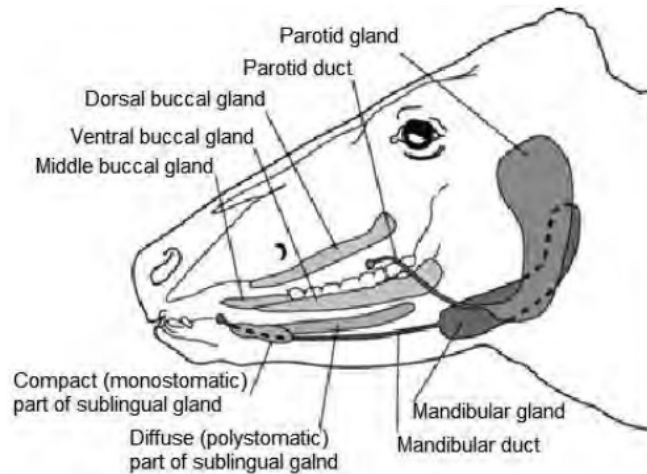


Fig. 2. Schematic summary of the distribution of salivary glands in ruminants (Source: <https://veteriankey.com/surgery-of-the-bovine-digestive-system/>)

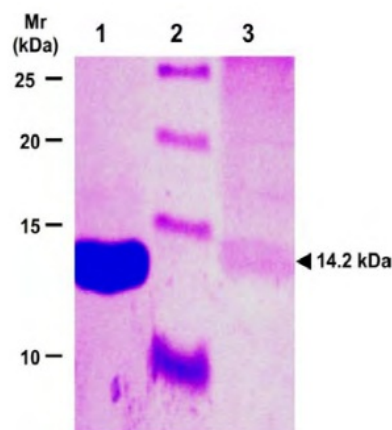


Fig. 3. SDS-PAGE profile of bioactive compounds isolated from Bali cattle (*Bos sondaicus/javanicus*) saliva by aqueous mixture of PEG4000/ Na_2SO_4 (lane 3); lane 1, Commercial hen egg white lysozyme; lane 2, molecular weight marker (Depamede et al. [11])

The compound was able to inhibit the proliferation of *Staphylococcus aureus* at twice the strength of a commercial hen egg white lysozyme which was $40 \mu\text{g}/\mu\text{L}$ vs $80 \mu\text{g}/\mu\text{L}$, respectively [11]. This shows that naturally, saliva plays an important role in maintaining the body's defence by providing multiple immunogenic components [12]. Furthermore, in regard to the role of saliva as medium to measure the process of food digestion and acting as a buffering agent, Depamede and Dahlanuddin [13] reported that saliva of Bali cattle can be used to measure the level of urea (saliva urea nitrogen, SalUN) as an indicator of diet quality given to ruminants comparable to those from blood serum or blood urea nitrogen (BUN).

3. PROTEOMIC AND MOLECULAR ANALYSIS OF BALI CATTLE SALIVA

We have also conducted studies on the proteins from Bali cattle saliva with a molecular weight of 14.2 kDa using MALDITOF-TOF Mass Spectrometer method [14]. The results of MALDITOF /TOF.MS

analysis with the help of Mascot search revealed that the 14.2 kDa protein is not a single peptide, instead at least three main protein sequences, with the nominal molecular weight range of 9-22 kDa (Tabel 1), Depamede [14]. The three peptides were namely G3MZ19 or also known as HRPE773-like Predicted zymogen granule protein 16 homologue B (*Bos taurus*), F1N1Z8 or Pancreatic adenocarcinoma upregulated factor-like (*Bos taurus*), and Predicted zymogen granule protein 16 homologue B (*Bos taurus*), and F1MVC8 or Prolactin-inducible protein homologue precursor (PIP-HP) of *Bos taurus*. It can be seen that all three peptides are related to the *Bos taurus* lineage, although Bali cattle itself is a descendant of *Bos Banteng* or *Bos javanicus* [1,2]. It is possible that because the proteomics of Bali cattle have not been widely studied, so the relevant information is not available, or it could also be related to the evolution of the three peptides. An interesting study needs to be disclosed further, especially in large ruminants.

If observed more closely, based on the nominal molecular weight of the three peptides, G3MZ19 with a molecular weight of 17.708 kDa is the closest to 14.2 kDa according to the SDSPAGE study. Furthermore, it should be noted that G3MZ19 is a member of the family of mannose-binding protein or mannose-binding lectin (MBL), which plays an important role in the innate immune system [15,16]. As far as our concerned, this was the first time revealed by Depamede [14] about the existence of G3MZ19 in the saliva of Bali cattle. The presence of G3MZ19 is very plausible because of one of the natural roles of saliva is as an initial guard for the entry of pathogens into the body via the oral cavity.

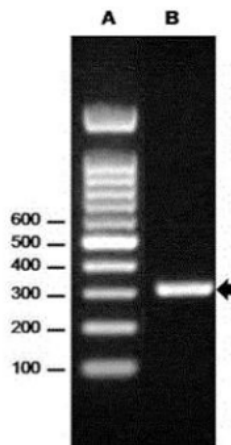


Fig. 4. Expression of G3MZ19 gene of 306 bp (B, arrow) in the Bali cattle saliva; A, DNA size standard (Reproduced from Depamede, [6])

Expression of G3MZ19 in the saliva of Bali cattle (*Bos javanicus*) which is genetically different from *Bos taurus*, has made researchers tempted to study it more deeply. Depamede [6] successfully studied the molecular sequences of G3MZ19 in Bali cattle. For this purpose, he used primers for the reactions ACGTACTATTGGATAATCCCAG and AGTATCCAGGTGAGGTTTGG as forward and reverse primers, respectively. The primers were retrieved from bovine *Bos Taurus* (G3MZ19 | LOC100295741, Tax_Id = 9913, LudwigNR database and UniProt). Depamede showed that genes or fragmented genes retrieved from *Bos taurus*, can be used to assess the presence of predicted zymogen granule protein genes in the Bali cattle genomic DNA. Expression of G3MZ19 gene with a size of 306 bp in Bali cattle saliva is presented in Fig. 4 [6].

4. THE POTENTIAL OF THE COMPONENTS OF BALI CATTLE SALIVA AS ANTI-BACTERIA

As stated earlier, Depamede et al. [11] reported that a compound sourced from Bali cattle saliva (MW 14.2 kDa) demonstrated its ability as a bactericide, surpassing the bacterial inhibitory properties of commercial hen egg lysozymes. Following this study, Depamede [14] elucidated that at least three

peptides instead of lysozymes were present in the 14 kDa compounds (Table 1). The interesting thing was that Kisworo and Depamede [17] reported that antibodies against prolactin-inducible protein homologue precursors (PIP-HP) of Bali Cattle (*Bos javanicus*) saliva was also able to inhibit the growth of both *Escherichia coli* and *Staphylococcus aureus* cultures significantly ($P < 0.001$) at a concentration of 0.3 mg/mL (Fig. 5). The antibodies were obtained by immunizing Indonesian local rabbits using a synthetic peptide fragment of PIP-HP (i.e. VIRELGICPDD WAVIPIKANRF), which had been coupled to bovine serum albumin (BSA) according to Grant [18], modified by Kisworo and Depamede [17].

According to Kisworo and Depamede [17] the ability of Anti PIP-HP polyclonal antibodies to inhibit both *S. aureus* and *E. coli* growth is quite interesting. Unfortunately, the information related to this matter is still limited. Previous researchers [19] who used different antibodies i.e. the egg yolk IgY of Ostriches, showed similar inhibition capacity toward the proliferation of *S. aureus* and *E. coli* significantly ($P < 0.05$). Meanwhile Ochsenbein et al., [20,21,22] reported that some natural antibodies have also been able to inhibit microbe's growth naturally. Kisworo and Depamede [17] then remarked that whether the Anti PIP-HP polyclonal antibody is in line with the mode of action of natural antibodies or it contributes to the antibacterial activities of the natural antibody repertoire as mentioned by Ochsenbein et al. [20], Zhou et al. [21], and Rothstein [22] need to be clarified further.

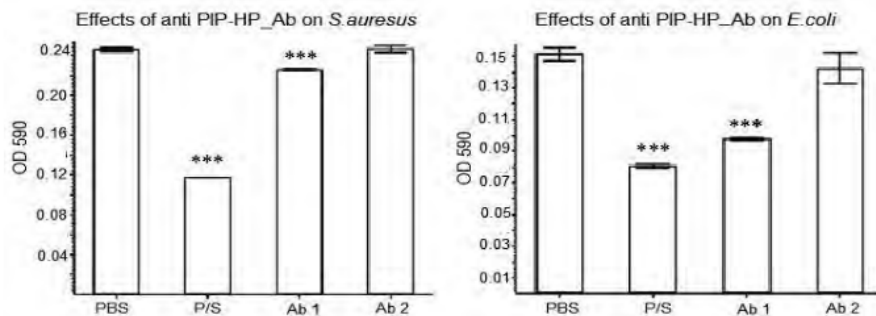


Fig. 5. Anti PIP-HP polyclonal antibody (Ab1 or Ab2) was able to inhibit both *S. aureus* and *E. coli* growth significantly ($***P < 0.001$) at concentration up to 0.3 mg/mL
PBS: Phosphate buffered saline, P/S: Penicillin (10 U/mL) & streptomycin (0.1 mg/mL),
Ab1 :0.3 µg/mL, Ab2: 0.3 µg/mL (Kisworo and Depamede, [17])

5. BOVINE SALIVARY PROTEIN 30b (BSP30b)

Up to this section, we have discussed more on the salivary proteins or peptides with molecular weights around 20 kDa or less. Salivary proteins with a molecular weight of about 30 kDa in bovine saliva have recently fascinated researchers' interest. Salivary protein with a molecular weight of 30 kDa, which later became known as bovine salivary protein 30 (BSP30) has been reported by Rajan et al. [23]. Furthermore, Wheeler et al. [24] have also classified BSP30 into four types, namely BSP30a, b, c, and d. BSP30a is a common form as found in whole *Bos taurus* saliva, while the forms of b, c, and d are scattered and vary among cattle.

In general, the BSP30 is closely linked to the susceptibility of bloating in *Bos taurus* cattle [25,26]. For some countries susceptibility of cattle to pasture bloat cannot be considered trivial since it has an impact both from the economic aspect as well as animal welfare issue. It has been reported that in New Zealand among dairy cattle in the low-susceptibility herd, BSP30 was found with relative abundance ($66\% \pm 15\%$ of 74 samples) compared to the susceptibility one.

Bloat prevalence data in Bali cattle is still limited. The interesting thing is that in Indonesia the occurrence of bloat due to feed grains or concentrates rarely occurs even though the maintenance system is largely deplored because of a lack of grazing land, a reversal of what is common in ruminants maintained in wards [27]. According to Yanuarto, the rare occurrence of bloat is due to the breeder's habit of adding molasses to wheat bran in their cattle rations [28].

2 Table 1. Mascot search results of protein spot (1-D 12.5% SDS-PAGE) of a 14.2 kDa isolated from Bali cattle saliva) identification by MALDI-TOF Mass Spectrometry (Taken from Depamede, [14])

Mascot hit number	Protein sequent ID	Score	Nominal mass (Mr, kDa)	Calculated pI	Protein sequence coverage (%)	Accession blast	Description	Max identity (%)
1	G3MZ19	425	17.708	8.52	48	gi 296473588 DAA15703.1 gi 359079571 XP_002697975.2	HRPE773-like (<i>Bos taurus</i>) Predicted zymogen granule protein 16 homolog B (<i>Bos taurus</i>)	100 100
2	F1N1Z8	131	22.091	9.41	13	gi 296473587 DAA15702.1.	Pancreatic adenocarcinoma upregulated factor-like (<i>Bos taurus</i>)	100
3	F1MCV8	79	9.007	8.96	25	gi 124249234 INP_001074382.1	Prolactin-inducible protein homolog precursor (<i>Bos taurus</i>)	99

Data on BSP30 in saliva of Bali cattle or cattle in Indonesia has never been reported so we cannot link between the low incidences of bloat in cattle in Indonesia with the presence of BSP30 in cattle in Indonesia in general and Bali cattle in particular. However, a preliminary study conducted by Depamede and Wheeler and his Team at AgResearch, Ruakura Research Centre, Hamilton, New Zealand showed that of the 25 samples of Bali cattle saliva examined, all of them expressed the presence of BSP30 (unpublished data). We then can speculate that in addition to the feeding system as mentioned by Yanuarto and his colleagues the presence of BSP30, especially BSP30b might play an important role in suppressing bloat incidences.

Many things still need to be disclosed regarding this BSP30b. Recently, Zhang et al. [29] have carried out a study on the three-dimensional structure of BSP30b and its interactions with specific rumen bacteria. According to them, BSP30b can bind to bacterial lipids of specific species and this profuse protein might have an important biological role through interactions with rumen bacteria during feeding and rumination.

6. FUTURE STUDY

From the description stated above, it has been clearly seen that much can be learned, but more is yet to be revealed from the saliva of Bali cattle. Bali cattle (*Bos javanicus*) as native Indonesian cattle with some superiority as tropical cattle seem to have considerable potential for further study. Its adaptability and resistance to heat stress and some tropical diseases are interesting enough to be studied more deeply through the use of saliva as a non-invasive biological material.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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