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Original Article
Livestock Diseases

'Beer yeast' (liquid brewery waste) for sheep and pig feeding: epidemiological and clinical aspects of the poisoning, safety margin and prevention[1]

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ABSTRACT. Aragão A.P., Brust L.A.C., Galvão A., Caldas S.A., Nogueira V.A., Santos B.B.N., França T.N. & Peixoto P.V. 2023. **'Beer yeast' (liquid brewery waste) for sheep and pig feeding: epidemiological and clinical aspects of the poisoning, safety margin and prevention.** *Pesquisa Veterinária Brasileira 43:e07112, 2023.* Universidade Federal Rural do Rio de Janeiro, BR-465 Km 7, Seropédica, RJ 23890-000, Brazil. E-mail: pfpeixoto19@gmail.com

Considering the practice of using 'brewer's yeast' (BY) – liquid brewery yeast waste from the brewing industry – in animal feed in some establishments in the south of the State of Rio de Janeiro in Brazil, this study was conducted to establish safety margins and to determine epidemiological and clinical aspects. In addition, this study suggested prophylactic measures that can prevent or minimise alcohol intoxication due to BY in sheep and swine. The main characteristics of the clinical features in both natural poisoning and controlled spontaneous ingestion in swine as well as intoxication by controlled ingestion of BY using a ruminal probe in sheep were found to be staggering gait, tripping and falls. Overall, 3.8 and 8.875mL/kg of ethanol content in the BY caused mild-moderate and severe clinical conditions, respectively, in swine and sheep. The following prophylactic measures are suggested: 1. BY must be adequately diluted in water, whey, or with BY that has been previously stored in the property (old BY); 2. It must be administered in proportion to the animals' weight and size. 3. It must be administered continuously, without interruptions, and with the provision of other food in the trough (such as soybean meal or cornmeal and water *ad libitum*). In conclusion, although BY is increasingly used in sheep and swine breeding properties – in the south of the State of Rio de Janeiro – many owners use one or more of the suggested prophylactic measures. Hence, ethanol intoxications because of BY are infrequent and rarely cause deaths; therefore, BY can be used provided adequate prophylactic measures are followed.

INDEX TERMS: Beer yeast, ethanol poisoning, sheep, swine.

RESUMO.- [**"Levedo de cerveja" (resíduo líquido de cervejaria) para alimentação de ovinos e suínos: aspectos epidemiológicos e clínicos da intoxicação, margem de segurança e prevenção.**] Considerando-se à prática de utilizar "levedo de cerveja" (LC) – resíduo líquido da indústria cervejeira – na alimentação de animais, em alguns estabelecimentos no sul do Estado do Rio de Janeiro, esse estudo foi realizado para estabelecer as margens de segurança e os quadros epidemiológico e clínico, bem como sugerir medidas profiláticas que impeçam ou minimizem esse tipo de intoxicação alcoólica para ovinos e suínos. Verificou-se que o quadro clínico na intoxicação por ingestão controlada de

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LC por sonda em ovinos e na intoxicação natural e ingestão espontânea controlada em suínos, caracterizou-se principalmente por andar cambaleante, tropeços e quedas. Concentrações de 3,8 e 8,875mL/kg de etanol contido no LC, causaram quadros clínicos leve a moderado e grave em suínos e ovinos, respectivamente. Como medidas profiláticas sugere-se: diluição adequada do LC com água, soro de leite ou com o LC já estocado na propriedade (LC antigo); administração do LC proporcional ao peso/tamanho dos animais, administração contínua, sem interrupções, disponibilizar outro alimento no cocho, como farelo de soja ou fubá de milho e água à vontade. Conclui-se que apesar de o LC ser cada vez mais utilizado nas propriedades criadoras de ovinos e suínos no sul do Estado do Rio de Janeiro já que, muitos proprietários aplicam uma ou mais medidas profiláticas aqui sugeridas, as intoxicações pelo etanol, contido no LC são pouco frequentes e raramente ocorrem mortes, de forma que esse produto deve ser utilizado, desde que as medidas profiláticas sejam observadas.

TERMOS DE INDEXAÇÃO: "Levedo de cerveja", intoxicação por etanol, ovinos, suínos.

INTRODUCTION

Owing to the need to reduce production costs given the seasonality and high price of production required for animal feed, such as soybean meal or cornmeal, a need for alternative foods has increased in recent decades. The use of agro-industrial by-products in beef cattle plays a significant economic and environmental role; in some areas, it is even responsible for maintaining the system's economic viability. In Brazil, residual 'brewer's yeast' (BY) from the brewing industry has previously been utilised in cattle feed. The solid portion of this by-product, popularly known as 'barley', has been successfully used to feed dairy cattle, particularly in the Southeast Region of Brazil (personal communication[8]); however, it can eventually become toxic when contaminated by *Aspergillus clavatus* (Bezerra Jr. et al. 2009). Moreover, it is important to consider that BY – a liquid brewery yeast waste from the brewing industry – may contain a significant amount of alcohol. In some rural properties of the State of Rio de Janeiro, cases of cattle intoxicated by ethanol contained in BY have been reported. The cattle that consumed this residue of BY as part of their diet eventually showed symptoms related to nervous system diseases. A proportion of affected animals died immediately, with some dying in less than 1 hour after ingesting large amounts of this by-product (Brust 2011).

Previously, BY has also been used for sheep and swine feeding in properties in the south of the State of Rio de Janeiro. It is important to emphasise that the by-products of the brewing industry are toxic and can cause serious problems if they are directly dumped into the environment. In addition to reducing production costs, the eventual use of these BY residues in animal feed can address the issues of environmental pollution associated with the brewing industry.

Research that accurately determines the safety margin in order to prevent cases of intoxication is required for the safe use of residual BY in animal feed. Therefore, the objectives of this study were as follows: 1) to determine the safe BY amount that can be fed to sheep and swine, 2) to suggest appropriate management of intoxication, 3) to identify the epidemiological and clinical aspects of intoxication in sheep and swine, 4) to collect the available data on the occurrence of BY-related intoxication in sheep and swine and 5) to propose measures for avoiding intoxication.

MATERIALS AND METHODS

Epidemiological and clinical survey

For the epidemiological survey and the study of the clinical aspects of acute and/or chronic intoxications, rural properties in the south of the State of Rio de Janeiro (Barra Mansa, Volta Redonda, Barra do Piraí and Porto counties), which used BY for feeding sheep and swine, were visited. The data and history were obtained from the veterinary, administrator of the properties.

Study of acute sheep intoxication

The administration of BY – controlled by a rumen probe – was conducted in a small property in the municipality of Porto Real. Five crossbred sheep were included, consisting of four males (Sheep 1, 2, 4 and 5; weighing 16, 12.5, 17 and 18kg, respectively) and one female (Sheep 3; weighing 28.5kg). The used BY batches were directly collected from the tank lorry that was used for transportation between the brewing industry and the rural properties. Thereafter, the BY was deposited in plastic gallons with a capacity of 50 litres. The time from BY collection to the administration was ≤4 hours.

The five sheep were clinically healthy, were previously treated for endo- and ecto-parasitic infections, were placed in paddocks measuring 2.5 × 4 metres, were adapted to the site and were routinely fed with concentrate, including chopped Guinea grass (*Megathyrsus maximum*) and water *ad libitum*. Before BY administration, all sheep fasted for 24 hours, and they were weighed and clinically examined (time zero). BY samples were collected (BY sample 1, administration 1; BY sample 2, administration 2). The samples were then stored in plastic bottles and frozen for later evaluation of the alcohol content.

After administration, the animals were periodically examined at different time points, particularly for the following parameters: neurological changes, posture, attitude, appetite, heart and respiratory rates, alcoholic odour in expired air, motility and degree of rumen distension, rectal temperature, frequency of urination and defaecation. Data regarding BY administration are shown in Table 1.

Study of acute swine intoxication

Spontaneous ingestion of uncontrolled BY (natural cases). In December 2007, we received information about a BY intoxication incident at a property in the municipality of Barra do Piraí, south of the State of Rio de Janeiro. A site visit was conducted to collect epidemiological and clinical data.

Spontaneous ingestion of controlled BY. The observation of spontaneous ingestion of BY was conducted on 11th June, 2009 at Property A, which was located in the municipality of Volta Redonda/RJ. In this property, swine, cattle and sheep were raised, and the BY was used for approximately 10 years. The swine were raised in collective pens that were categorised into batches according to age and breed. The BY used in the rural property was obtained daily from two tank lorries, and it was stored in tanks with a total capacity of 240,000 litres.

[8] Information provided by Pedro Muniz Malafaia, MV Dr., Department of Animal Nutrition and Pastures, Instituto de Zootecnia, UFRRJ, 2007.

In the property, all swine received BY as well as soya bean meal or cornmeal daily *ad libitum* in a separate trough in addition to the water in pacifier-type drinkers.

The following five batches were observed: Batch A comprising of eight hybrid piglets with the age of 40 days and an average weight of 30kg; Batch B comprising of seven NDB (non-defined breed) piglets with the age of 40 days and an average weight of 2-5kg; Batch C comprising of four hybrid piglets with the age of 30 days and an average weight of 20kg; Batch D comprising of a pair of hybrid breeders with the age of 5 months and an average weight of 60kg.

Before administration, animals from all batches (except for Batch D) were weighed and fasted for 24 hours. BY was administered in the same manner as it had been administered every day, i.e. by placing BY in the troughs of each batch using plastic buckets with an average individual capacity of 20 litres. After the administration and beginning of ingestion, the animals were observed, particularly for the neurological changes, posture, attitude and appetite.

Laboratory analysis of ethanol levels in the BY

BY samples 1 (administration 1, sheep), 2 (administration 2, sheep) and 3 (sample used with the alcoholometer) were stored in plastic bottles, frozen and sent to the Analytical Laboratory of Food and Beverages/LAAB-UFRRJ for assessing alcohol content. A part of BY sample 2 was separated, frozen and thawed for 24 hours before using the alcoholometer (Densimeter for alcohol 0%/10% by volume, calibrated at 20°C, according to Gay Lussac). The sample obtained from this filtering was used as BY sample 3.

RESULTS

Properties

Property A: Municipality of Volta Redonda/RJ. The data were provided by the responsible veterinary and the administrator or owner during visits conducted on 11th June, 2009 and 3rd November, 2011.

When sheep ingested excess BY, they presented signs of drunkenness and died. The symptoms began to appear 30 minutes after the start of consumption, and the symptoms were described as staggering gait, atypical positions, falls and drowsiness. The swine received BY *ad libitum* once a day in the trough. They also received soya bean meal or cornmeal *ad libitum* in a separate trough and water in pacifier-type drinkers.

The swine began ingesting the residual BY at the age of 28 days (after weaning), and they consumed it until slaughter (i.e. 4-5 months). The average daily consumption of BY was 2.5 litres – always diluted in water – per animal. When symptoms of drunkenness (such as staggering gait) appeared, the swine more intensely searched for cornmeal, although they did not stop drinking BY. Deaths following BY ingestion were mentioned. The owner was unable to provide

Table 1. Main data on controlled administration of brewer's yeast (BY) using probe in sheep

Animal	Weight	Alcohol content of BY	Dose/Dosage of BY	Onset of symptoms	Type of intoxication	Main clinical findings	Clotting time
Sheep 1	16kg	7.1%	2L/125mL/kg	20 minutes	Severe	Shocks against obstacles, slight hypermetry, crossing of limbs and atypical postures, such as the abduction of the limbs and delay in their correction, were observed; the condition evolved to trips, falls and even a momentary inability to get up. During grazing, the sheep showed instability and staggering. As the condition progressed, the animal fell asleep and took a long time to wake up even after external stimulation. In recumbency, the animal could not support its head and presented with mild–moderate salivation and moderate bloating; it discreetly gritted the teeth with eructation whenever manipulated during the clinical examination	18 h
Sheep 2	12.5kg	7.1%	1.5L/120mL/kg	10 minutes	Severe	Fell into lateral recumbency, was unable to get up and did not respond to external stimuli. Moreover, moderate–severe nystagmus, mild salivation and moderate bloating were observed; the animal belched, urinated and gnashed its teeth during the clinical examination. Within 18 hours and 15 minutes of progression, the sheep began to respond to external stimuli; however, it could not remain stationary (limbs without tone).	24 h
Sheep 3	28.5kg	3.4%	3.5L/123mL/kg	41 minutes	Mild	Gritting of the teeth, mild–moderate apathy, alterations in the positioning of the limbs, mild instability progressing to tripping, falls and difficulty in getting up. The animal maintained normal appetite and urinary excretion.	7 h
Sheep 4	17kg	3.4%	2.5L/147mL/kg	50 minutes	Moderate	Gritting of the teeth, mild apathy, mild nystagmus that worsened in the lateral decubitus position, alterations in the positioning of the limbs, accentuated instability progressing to trips and falls with momentary inability to get up. In the stationary position, the sheep moved its head towards the flank; however, when stimulated to walk, the sheep's head returned to the normal position.	7h
Sheep 5	18kg	3.4%	2L/125mL/kg	13 minutes	Mild to moderate	Gritting of the teeth, moderate apathy, mild nystagmus, alterations in the positioning of the limbs, moderate instability progressing to tripping and falls; however, difficulty in getting up was not observed.	6 h

specific information on clinical or necropsy data, although the property's employees always used the meat for their personal meals. Notably, deaths occurred only when BY was administered freshly in an undiluted form and if the animal did not ingest BY for at least 1 day. The procedure used in in cases of alcohol poisoning consisted of bathing in cold water and encouraging the ingestion of cornmeal and water in addition to prohibiting the ingestion of BY until complete improvement. The earlier this procedure is initiated, the greater the chance of recovery.

Property B: Municipality of Barra Mansa/RJ. The information was provided by the administrator during a visit conducted on 3rd November, 2011. According to history, this was the first property in the State of RJ to use this brewery by-product in animal feed in 1987.

The sheep (Texel breed) showed no symptoms of drunkenness even when they were confined to receive BY *ad libitum* in the trough or were not ingesting BY for some time. The sheep were raised for a long time together with beef cattle in *Brachiaria* pastures with free access to BY troughs.

The swine (crossbred of Caruncho Piau) were raised in confinement and received BY *ad libitum* once a day in the trough; they also received cornmeal *ad libitum* in a separate trough and water in pacifier-type drinkers. The swine began ingesting the BY after weaning (for an average of 30 days) and drank it until slaughter (i.e. approximately 5 months). As the consumption was *ad libitum*, the property manager did not know the average daily consumption per animal. Only a few symptoms of mild-moderate intoxication, such as staggering gait, drowsiness and – rarely – lateral recumbency, were reported; however, no major problems or the need for intervention were reported. No deaths caused by the ingestion of BY were reported.

Property C: Municipality of Barra Mansa/RJ. The information was provided by the handler during a visit conducted on 3rd November, 2011.

The sheep (NDB) of the property were freely raised together with the cattle, with free access to the troughs containing BY; however, drunkenness-related symptoms or deaths due to the consumption of BY were not reported.

The swine (NDB) received BY *ad libitum* once a day in a trough, feed and bread in a separate trough, and water in pacifier-type drinkers. The swine were raised in confinement, and they ingested BY from 40 days of age to the day of slaughter (i.e. 6-7 months of age). The average daily consumption of BY diluted in whey per animal was 4 litres. Significant weight gain was observed in the swine after the initiation of BY use in the feed. It was reported that when some symptoms of drunkenness (such as staggering gait) appeared, the swine more intensively searched for feed and drank more water; however, they did not stop drinking the residual BY. No deaths due to BY consumption were observed in this property.

Property D: Municipality of Barra do Piraí/RJ. The information was provided by the veterinary during a visit conducted on 3rd November, 2011.

The sheep (Santa Inês breed) did not receive BY, but they had free access to the bovine troughs; thus, they ingested the BY residual daily. No drunkenness-related symptoms or deaths due to the consumption of BY were reported.

The swine (Java pig) were raised in the property for personal consumption. The swine received BY *ad libitum* once a day in the trough; they also received cornmeal and water in pacifier-type drinkers. Although BY consumption was free, the animals did not show symptoms of intoxication, and deaths were not reported.

Clinical data

Controlled administration using a ruminal probe in sheep: Mild acute alcohol intoxication. The administration was conducted in the Porto Real property. The Sheep 3 was fasted for 24 hours before the administration of 3.5 litres (123mL/kg) of BY using a ruminal probe. An immediate moderate enlargement of the left paralumbar fossa was observed. At 10 minutes after administration, hiccups were observed; however, the attitude and appetite remained normal. The intoxication that was classified as mild had the onset of symptoms at 41 minutes after administration, and it was characterised by gritting of the teeth, mild-moderate apathy, alterations in the positioning of the limbs, mild instability progressing to tripping, falls and difficulty in getting up. The animal maintained normal appetite and urinary excretion. After 7 hours of administration, the animal showed an alert attitude and locomotion without any change.

Mild-moderate acute alcohol intoxication. The Sheep 5 was fasted for 24 hours before the administration of 2 litres (125mL/kg) of BY using a ruminal probe. An immediate remarkable increase in the left paralumbar fossa was observed, and at 3 minutes after the administration, hiccups were observed. The intoxication classified as mild-moderate had the onset of symptoms at 13 minutes, and it was characterised by gritting of the teeth, moderate apathy, mild nystagmus, alterations in the positioning of the limbs, moderate instability progressing to tripping and falls; however, difficulty in getting up was not observed. The animal maintained normal appetite and urinary excretion. After 6 hours of administration, the animal showed an alert attitude and locomotion without any change.

Moderate acute alcohol intoxication. The Sheep 4 was fasted for 24 hours before the administration of 2.5 litres (147mL/kg) of BY using a ruminal probe. An immediate moderate enlargement of the left paralumbar fossa was observed. The intoxication classified as moderate had the onset of symptoms in 50 minutes, and it was characterised by gritting of the teeth, mild apathy, mild nystagmus that worsened in the lateral decubitus position, alterations in the positioning of the limbs, accentuated instability progressing to trips and falls with momentary inability to get up. In the stationary position, the sheep moved its head towards the flank; however, when stimulated to walk, the sheep's head returned to the normal position. The animal maintained normal appetite and urinary excretion. After 7 hours of administration, the sheep recovered.

Severe acute alcohol intoxication. Sheep 1 and 2 were fasted for 24 hours before the administration of 2 litres (125mL/kg) and 1.5 litres (120mL/kg) of BY using a ruminal probe, respectively. An immediate moderate increase in the left paralumbar fossa was observed in the animals. In these cases, the intoxications were severe, and symptoms began to develop within 10-20 minutes. At 10 minutes after ingestion, Sheep 2 fell into lateral recumbency, was unable to get up and did not respond to external stimuli. Moreover, moderate-severe nystagmus, mild salivation and moderate bloating were observed; the animal belched, urinated and

gnashed its teeth during the clinical examination. Within 18 hours and 15 minutes of progression, the sheep began to respond to external stimuli; however, it could not remain stationary (limbs without tone). Complete recovery was observed within 24 hours of the onset of clinical signs. In Sheep 1, 20 minutes after BY administration, shocks against obstacles, slight hypermetry, crossing of limbs and atypical postures, such as the abduction of the limbs and delay in their correction, were observed; the condition evolved to trips, falls and even a momentary inability to get up. During grazing, the sheep showed instability and staggering (Fig.1 and 2). As the condition progressed, the animal fell asleep and took a long time to wake up even after external stimulation. In recumbency, the animal could not support its head and presented with mild-moderate salivation and moderate bloating; it discreetly gritted the teeth with eructation whenever manipulated during the clinical examination. The main data verified in the controlled administration are described in Table 1.

Fig.1. 'Beer yeast' (liquid brewery waste) for sheep and pig feeding: epidemiological and clinical aspects of the poisoning, safety margin and prevention. Severe intoxication. The onset of symptoms with staggering gait and standing in atypical positions. Sheep 1; 2L dose of brewer's yeast.

Fig.2. 'Beer yeast' (liquid brewery waste) for sheep and pig feeding: epidemiological and clinical aspects of the poisoning, safety margin and prevention. Severe intoxication. The progression of the condition with a fall in the lateral decubitus position, permanence in an atypical position and deep sleep. Sheep 1; 2L dose of brewer's yeast.

Natural poisoning in swine

Natural cases of acute ethanol intoxication in swine were reported in December 2007, in the municipality of Barra do Piraí, Rio de Janeiro. The farm's swine were routinely fed with a mixture of 50kg of barley added to 20L of leftover feed and 10kg of wheat bran only once a day in the morning. The lorry that typically transported BY was transporting barley on the day of the intoxication; it was considered that a mixture of barley and BY might have formed because the barley was more liquid and had a strong BY odour. The barley (which had more liquid and was probably mixed with BY) was fed to all swine. After ingestion, the piglets observed on the next day morning showed symptoms of drunkenness, and in the afternoon, one of them was found dead. On the subsequent day, the piglets received the same food. Approximately 1 hour after the start of ingestion, seven piglets aged approximately 5 months were active. However, they had mild-moderate motor incoordination, muscle tremors and frequent urination; while moving or running, some walked backwards and sat on hind legs or fell, rolled over and had difficulty getting up; and some animals presented with vomiting. On the next day, all piglets recovered and had no symptoms of drunkenness. To avoid further intoxications, it was recommended to dilute the barley in water; therefore, no new cases occurred. The same mixture that intoxicated the piglets was fed to dairy cattle, which did not show any symptoms. A sample of barley provided to the swine on the day of intoxication was analysed at LAAB/UFRRJ, and it revealed a total alcohol content of 2.7%, including 2.54% acetaldehyde, 0.15% ethanol and 0.01% of other contents.

Controlled spontaneous administration in BY swine

Clinical observation of BY intoxication in swine was performed during a visit to a rural property (Property A) located in the municipality of the Volta Redonda/RJ.

Batch A. Batch A consisted of eight swine with an average weight of 30kg and an age of approximately 40 days. The swine were subjected to 24 hours of fasting. Overall, 13 litres of BY was administered at 12:12 p.m. Three female swine did not want to ingest the BY; however, the remaining five ingested it with great avidity, and at an average of 20 minutes after the beginning of ingestion, they showed signs of intoxication. Within 30 minutes, the five animals showed instability during walking, signs of drowsiness, falls and – less frequently – lateral recumbency, difficulty in getting up and frequent urination.

Batch B. Batch B consisted of seven NDB piglets with an average weight of 2-5kg and an age of approximately 40 days (they were breeding animals in the property). Overall, 13 litres of BY was placed in the trough without dilution at 12:12 p.m. The seven animals avidly ingested the residual BY; at 10-20 minutes after the beginning of ingestion, some swine showed signs of drunkenness and searched for the trough of soya bean meal and water. Within 28 minutes of progression,

most animals already showed instability while walking and falls. Some showed signs of aggression, i.e. charged at others, chased others and bit their ears (Fig.3). Other signs observed were drowsiness (standing or lying down), falls, periods of lateral recumbency, difficulty in getting up, atypical positions, frequent urination and muscle tremors (Fig.4).

Batch C. Batch C consisted of four animals with an average weight of 20kg, they received 13 litres of undiluted BY at 12:14 p.m. They did not ingest the soya bean meal in the trough. The four animals already showed signs of intoxication at 9 minutes after the beginning of the ingestion, characterised by staggering, falls, difficulty in getting up, slips, instability, drowsiness (prostrate with the head down) and – sometimes – sitting dog posture. Urination and water intake became more frequent, and some animals, even those who were intoxicated, returned to the trough and ingested more BY. The largest piglet in the batch presented with a sitting dog posture and remained in the lateral position after 1 hour and 47 minutes. It stood up 32 minutes later, still staggering, to ingest BY again; however, it again fell into the lateral position inside the trough and slept soundly. Twenty-two minutes later, still in lateral decubitus position and trembling, it had a temperature of 37.7°C and a heart rate of 132 bpm.

Batch D. Batch D included a couple with an average weight of 60kg and an age of 5 months that was not subjected to previous fasting. At 12:14 p.m., both the animals received 20 litres of BY diluted in seven litres of water in the trough; they ingested BY immediately and with great avidity. Accentuated signs of drunkenness, such as staggering, falls in atypical positions and difficulty in getting up at 31 minutes after ingestion, were observed, especially in the female (Fig.5). The male frequently experienced lateral recumbency or was on top of the female, making it difficult for the latter to breathe. Three hours after intake, BY reflux was detected through the nostrils. The male also exhibited respiratory rales, strong instability, and falls. The animal stood up when touched without much effort, staggered while moving and insisted on rousing the female. Within 15 hours of progression, the female was sleeping in the lateral decubitus position. After being insisted upon, the female was moved to the sternal decubitus position. The female then sat down and, with assistance, managed to stand up. The female then moved around the stall, with very much tottering, remaining in the station with difficulty and, after a few minutes, fell back down and slept again.

Fig.3. 'Beer yeast' (liquid brewery waste) for sheep and pig feeding: epidemiological and clinical aspects of the poisoning, safety margin and prevention. Piglets showing signs of aggression, charging at others, chasing others and biting their ears. Batch B.

Fig.4. 'Beer yeast' (liquid brewery waste) for sheep and pig feeding: epidemiological and clinical aspects of the poisoning, safety margin and prevention. Piglets sleeping in atypical positions. Batch B.

Alcohol content

Laboratory analyses of BY. The analysis of ethanol levels in BY samples 1, 2 and 3 revealed 7.1, 3.4 and 2.0% of alcohol content, respectively.

Use of an alcoholometer (Densimeter). To establish an empirical method to help the rural producers to estimate the alcohol content of BY, we used an alcoholometer (Densimeter for alcohol 0%/10% by volume, calibrated at 20°C, according to Gay Lussac).

DISCUSSION

An epidemiological study of BY use

During our clinical-epidemiological survey, we found that several properties in the south of the State of Rio de Janeiro used BY (the liquid brewery yeast waste) in animal feed. However, it was predominantly used for raising cattle, both beef and dairy, as described by Brust (2011). Moreover, in the properties under study, it was observed that sheep farming was not the main economic activity, but it was only a parallel activity for personal consumption and/or eventual sales. Therefore, the use of BY was generally intended for other animal species, mainly cattle and swine. This reduced

Fig.5. 'Beer yeast' (liquid brewery waste) for sheep and pig feeding: epidemiological and clinical aspects of the poisoning, safety margin and prevention. Female sleeping in an atypical position after a fall. Batch D.

the possibility of intoxication and losses in sheep. Notably, in all properties, the sheep had free access to the bovine trough, and in Property B, the animals were daily fed with BY; however, no clinical case of drunkenness was reported in this species. Thus, health problems due to the use of BY for sheep feeding may not be significant because natural cases of intoxication with progression to death in this species are rare, as reported only in Property A.

In the clinical-epidemiological survey on the use of BY in the State of Rio de Janeiro by Brust (2011), it was reported that there were occasional outbreaks of acute intoxication by ethanol (contained in BY) in cattle, including the deaths of animals. According to a survey, although the occurrences of deaths are rare, the incidence of intoxication in swine appears higher than that in sheep. Therefore, greater care should be taken in this species. In the properties under study, we did not find any case of chronic intoxication in sheep or swine; this can be explained by the type of production and the lifespan of these animals. For example, in the properties, sheep were not raised using BY (milk or beef), and swine were slaughtered after ≤8 months, thereby leaving insufficient time for developing chronic intoxication. In humans, among other conditions, chronic alcohol intoxication is the condition that leads to liver cirrhosis, and it only occurs after several years of alcoholism. None of the owners reported this type of liver injury in the slaughtered animals. Thus, Brust (2011) observation can be supported because no changes in the liver were seen in 50 cattle that ingested BY freely and continuously for 3-4 months; only one bovine, which ingested BY for 7 months, showed mild-moderate steatosis but no liver cirrhosis. The economic and financial benefits and methods to prevent intoxication are two other important factors to be considered during the use of BY in animal feed. The advantages of using BY in sheep and swine feed are evident, considering the opinions of the owners. Given what was seen in Property A, where a weight gain of 3-4kg/month was reported with a carcass yield of 45% for beef sheep of the Santa Inês breed, the cost was low, and the weight gain was excellent. For cattle, this beneficial effect of including BY in the diet has already been established (Brust 2011). Regarding the prophylaxis of intoxication, a way to avoid intoxication is the dilution of BY. Although most owners do not specify the dilution ratio in water, whey or old BY (with ethanol evaporation), they all agree that dilution reduces the risk of intoxication as new BY has high-efficiency fermentation and consequently higher alcohol content. Similar to the results of the present study, the epidemiological survey of ethanol (contained in BY) intoxications by Brust (2011) states that strong and undiluted newcomer BY or lack of experience in handling by-products are important factors in intoxication outbreaks. Moreover, Hibbs et al. (1984) stated that an error during handling of feed with by-products (derived from the production of fuel alcohol) was responsible for an outbreak of intoxication in confined calves. According to the practices conducted in Property C, the intake of ≤4L/BY/day diluted in whey is safe, at least for the animals closest to slaughter, if this is considered the average daily intake of the property. Hence, increasing amounts of BY should be made available according to the weight of the swine. When the average consumption was 4L/BY/day, some swine (the larger ones) ingested much more than that, whereas other swine (the smaller ones) – aged 1 month – received smaller amounts. In Property A, five-month-old piglets consumed an average of 2.5L/BY/day. All interviewers mentioned that the daily use of BY in sheep and swine feed resulted in excellent and rapid weight gain and carcass yield, among other benefits. According to the literature, calves that had been continuously intoxicated with ethanol showed weakness and weight loss (Abe et al. 1971). Moreover, preventing swine from receiving fresh undiluted BY and spending periods without receiving it can be considered as prophylactic measures to prevent the condition, similar to cattle (Brust 2011), that favours greater ingestion and intoxication as reported in Property A.

Acute alcohol poisoning in sheep

Occurred due to the administration of BY in sheep, and the alcohol content present in this residual BY was evaluated. As no data were available on the amount of BY that a sheep could ingest under natural conditions, the amounts were determined considering the animal weight and rumen capacity (Getty 1986). After the administration of BY using a ruminal probe, there was an immediate increase in the left paralumbar fossa due to the single administration of a large amount of liquid. Two hours after the clinical course, mild-moderate gas bloat was observed. For example, Sheep 1 and 2 showed moderate bloat, and they only belched with manipulation during clinical examinations. Previously, the same type of bloat has been described in cattle severely intoxicated by BY (Brust 2011). Ruminal gas bloat is a common condition in ruminants that is caused by excess gas formation or difficulty in eliminating gas (Radostits et al. 2002). There are varying degrees of abdominal distension (Clarke & Reid 1974) due to ruminal gas bloat, and deaths are a consequence of reduced lung capacity and hypoxia resulting from rumen pressure on the diaphragm (Radostits et al. 2002). The extent of interference with the microbial–biochemical processes of digestion in the pre-stomachs by the fermentative action of the yeasts contained in BY, mainly due to the changes caused by the residual BY during the storage time, remains unknown (Brust 2011). However, in our studies, with administration controlled by ruminal probe, the cases of bloat were apparently caused by the difficulty of eliminating the gas produced in the rumen due to the effect of alcohol. Clinical signs of drunkenness, which ranged from mild to severe, were very similar to those described in cattle that were naturally or experimentally intoxicated by BY (Brust 2011, Trujillo et al. 2018) or ethanol (Abe et al. 1971, Hibbs et al. 1984, 1986, Wijayasinghe et al. 1984, Stöber 2005). Thus, as reported by Brust (2011), during severe intoxication (Sheep 1 and 2), there was a remarkable flaccidity or muscle relaxation with total inability of the animals to remain stationary. The described signs were mainly characterised by drunkenness, accompanied by tremors, staggering, ataxia, stumbling, falls, standing or lying in atypical positions, drowsiness and alcoholic odour in expired air, as described by Brust (2011). Generally, as shown in Table 2, there was some relationship between the clinical aspects of intoxication (with the amounts of ethanol ingested) and the weight of the animal. However, age may influence the severity of the intoxication condition as Sheep 5 manifested with mild-moderate intoxication despite having ingested a smaller amount of ethanol (3.8mL/kg) than Sheep 3, which manifested with only mild intoxication (4.176mL/kg).

Table 2. Relationship between the clinical aspects of controlled brewer's yeast administration using a probe with the amount of ethanol ingested and the weight of the animal

Animal	Weight (kg)	Total dose of ethanol (mL)	Dose of ethanol per kg	Age	Clinical condition
Sheep 1	16	142	8.875 mL	5 months	Severe
Sheep 2	12.5	106	8.48 mL	5 months	Severe
Sheep 3	28.5	119	4.176 mL	1 year and 6 months	Mild
Sheep 4	17	85	5 mL	1 year and 2 months	Moderate
Sheep 5	18	68	3.8 mL	1 year	Mild to moderate

According to the results of a study by Brust (2011), with the administration of BY using ruminal probe, Bovine 1, weighing 130kg, presented with mild intoxication after the ingestion of 5 L of BY with 5% ethanol content, accounting for a total ingestion of 250mL of ethanol (1.923mL/kg). Bovine 2, weighing 145.5kg, showed severe intoxication after ingesting 10L of the same BY sample, accounting for a total ingestion of 250mL of ethanol (3.44mL/kg).

The two sheep in our study that received BY via a rumen probe showed severe intoxication that lasted from 6:20 to 11:45 hours for complete recovery. Abe et al. (1971) stated that in calves intoxicated by ethanol through the intestinal fistula, the symptoms started from 30 to 90 minutes of administration and regressed within ≥3 hours in severely intoxicated animals. Sheep 1, 2 and 3 showed geophagy during the final phase of recovery. We have no explanation for this behaviour.

Acute alcohol poisoning in swine

The diagnosis of natural poisoning in this species was based on clinical-epidemiological findings and the reproduction of the condition through the administration of the same food. Ethanol intoxication occurred in piglets that received barley, which was unintentionally mixed with BY during lorry transport. The clinical signs of drunkenness, such as ataxia, incoordination, falls, drowsiness or deep sleep, were similar in natural cases and in those with controlled spontaneous ingestion. Among the supervised swine, the piglets that spontaneously ingested BY in the trough showed aggressive behaviour (Batch B). Becker et al. (1954) reported that ethanol intoxicated swine with diets containing 25% and 50% glucose, showed incoordination, followed by rapid recovery. However, we found that the signs of intoxication lasted for an average of 12 hours, and the recovery of all animals was slow and gradual.

Safety margins and prophylaxis

Based on the epidemiological survey and the observations of intoxications verified in the current study, the use of BY in sheep and swine feeding can be considered safe if: I) BY is diluted in water, whey or in old BY; II) Administration of BY is proportionate to weight; III) There is continuous administration, without interruptions; IV) The ethanol from BY was evaporated (old BY); V) Other food is made available; VI) Water is available *ad libitum*.

It is important to remember that varying ethanol concentrations may be present in BY. Concentrations of ethanol fluctuated between 2%, 3.4%, 7.1%, and 5% in the analyses conducted in this study and that by Brust (2011). Notably, the BY storage tanks on these properties were often frail structures without lids, which may have caused the contamination of the BY residual with, for example, animal carcasses. One of the visited properties had a bird carcass that was already decomposing in the trough with BY, which poses a concern for botulism. Additionally, cases of intoxication by *Aspergillus clavatus* by the consumption of contaminated malt bagasse were mentioned by Bezerra Jr. et al. (2009).

Differential diagnosis

Some conditions must be considered in the differential diagnosis. Diseases that cause neurological symptoms and occur in the same region, such as rabies, botulism, ruminal acidosis, ketosis and intoxication by *Aspergillus clavatus*, must be differentiated from the condition caused by BY intoxication.

Rabies in cattle and – rarely – in sheep has an incubation period that varies from 2 weeks to several months, and it always has a fatal outcome with a progression period between 2 and 10 days. The clinical aspects of cattle and sheep are very similar, characterised by excessive salivation, behavioural modification, the instability of the hind limbs, aggression, flaccidity of the tail, tenesmus with paralysis of the anus, incoordination, permanent recumbency, opisthotonos and death (Radostits et al. 2002, Smith 2006). In swine, rabies manifests with excitement and a tendency to aggression, dullness and incoordination. Affected female swine may experience spasmodic twitching of the snout, rapid chewing movements, excessive salivation, clonic convulsions and backward walking. In the terminal form, paralysis is commonly seen, and death occurs within 12-48 hours after the onset of signs. The definitive diagnosis is made using the histopathological examination of the central nervous system, which reveals non-purulent encephalitis and the presence of Negri corpuscles; a positive immunofluorescence test (Radostits et al. 2002) or immunohistochemistry.

Epizootic botulism usually occurs in the form of outbreaks due to ingestion of food contaminated with carcasses containing botulinum toxin (Radostits et al. 2002). In Brazil, they are usually related to phosphorous deficiency as animals ingest bones (Tokarnia et al. 2010). Symptoms begin between 1 and 17 days after access to the toxic material with clinical manifestations of flaccid muscle paralysis, difficulty in locomotion, tongue, mastication and swallowing paralysis and absence of macroscopic or microscopic findings. The diagnosis must be made considering the history, symptoms and detection of the toxin by immunodiffusion in gel (Fernandes 2001).

Ruminal acidosis is a disease associated with the ingestion of a diet containing excessive highly fermentable carbohydrates (Schild 2001). This disease causes varying degrees of central nervous system depression and muscle weakness. The major symptoms are uncoordinated gait; frequent falls; bloat; reduced movement and even ruminal atony, sternal recumbency and coma (Radostits et al. 2002). Animals that become intoxicated after the consumption of BY may show some similarities with the typical signs of indigestion; however, the differentiation

does not pose major issues as cattle with ruminal acidosis continue to manifest with severe dehydration, enophthalmos and hemoconcentration; softened or diarrheal faeces with bittersweet or malodorous odour; and specific changes in the rumen fluid examination (Dirksen 1981, 2005).

Ketosis is a multifactorial disorder of energy metabolism that can occur at different stages of the pregnancy-lactation cycle in cattle and sheep. It affects cows with good body condition and a high potential for milk production, especially in the first month of lactation. In sheep, it is associated with a failure in the nutritional plan, particularly in the last month of pregnancy, in females that give birth to twin or triplet lambs, and it is associated with a failure in the nutritional plan due to other causes of stress. The main metabolic disorders observed are hypoglycaemia and acetonemia. Clinically, the two types of ketosis are as follows: wasting, which is characterised by a gradual decrease in appetite, weight loss, a characteristic odour of acetone in the breath and milk and a drop in milk production; nervous form, in which the animal walks in circles; crosses its legs; presses its head against objects; and presents with blindness, aimless movements, vigorous licking of the skin, depraved appetite, masticatory movements with salivation, tremor, uncoordinated gait and convulsions. Neurological symptoms are usually observed in short episodes, lasting from 1 to 2 hours, and may recur at approximately 8-12 hour intervals. The diagnosis is made using clinical pathology that reveals ketonaemia, hypoglycaemia, ketonuria and high concentrations of ketone bodies in milk. In sheep, high concentrations of beta-hydroxybutyrate are found in the aqueous humour of dead animals (Radostits et al. 2002).

The consumption of by-products from breweries or distilleries contaminated by the fungus *Aspergillus clavatus* has been associated with neurotoxicosis in cattle and sheep in several countries (Kellerman et al. 1976, 1984, 2005, Gilmour et al. 1989, Sabater-Vilar et al. 2004), including Brazil (Loretti et al. 2003, Bezerra Jr. et al. 2009, Oliveira 2016). The progression is longer than BY intoxication, and the main symptoms are characterised by hyperaesthesia, muscle tremors, ataxia, walking on the fetlocks of pelvic limbs, paresis and progressive paralysis. Animals that survive for long periods may show, at necropsy, pale areas in the skeletal muscles of the pelvic and thoracic limbs. In some cases, they also present with pale foci in the heart (Kellerman et al. 2005). Microscopically, there is degeneration and necrosis of large neurons, particularly the ventral horns of the spinal cord, specific nuclei of the medulla oblongata, midbrain, thalamus and spinal ganglia (Kellerman et al. 2005, Bezerra Jr. et al. 2009).

In addition to economic and nutritional factors, the importance of using BY residual in animal feed is related to the resolution of a significant and ongoing issue for the brewing industry, i.e. minimising environmental pollution and reducing costs for treating a part of these BY residuals. Notably, there have been no studies on the actual nutritional value or cost-effectiveness of using this type of BY residue in animal feed, and there are a few studies on the amount of macro- and microelements it contains.

CONCLUSIONS

Despite the brewery's liquid by-product – 'brewer's yeast' (BY) – being increasingly used in sheep, swine and cattle breeding properties in the southern region of the State of Rio de Janeiro, ethanol (contained in BY) intoxications are few and deaths rarely occur.

Hence, this product can be used provided the prophylactic measures, such as adequate dilution of BY in water, whey or with old BY (ethanol evaporation); administration of diluted BY proportional to the weight or size of the animals; continuous administration without interruptions; dilution or waiting for the ethanol to evaporate (old BY) when BY shows high fermentative activity; making other food available (such as soya bean meal or cornmeal and water *ad libitum*), in the trough, are followed.

In addition to promoting a reduction in feed costs, the use of BY in animal feed can minimise environmental pollution issues associated with this type of industry.

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