Substance P concentrations in the blood plasma and serum of adult cattle and calves during different painful procedures and conditions – a systematic review

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Abstract

Background: Pain in cattle is a major welfare problem, as cattle mask their pain. Subjective and objective parameters to assess pain in cattle have been described. Among the objective parameters to evaluate pain in cattle is substance P (SP). SP is a neurotransmitter, which is involved in the processing of noxious information to the brain; it seems to be a more objective indicator for nociception than cortisol, which has long been used as a biomarker for pain and stress in cattle. The objective of this systematic review was to assess the existing literature about SP during painful procedures, conditions, and diseases in cattle in form of a systematic review.

Results: Following the PRISMA statement, 36 out of 236 studies were included in this systematic review. Study design, grouping, age and weight of animals, processing of blood samples for the assessment of SP, and results were heterogeneous. The largest number of studies originated from the United States of America and Canada and were published in 2018. A higher number of studies were done on calves (69.4%, n = 25) compared with adult cattle (30.6%, n = 11). Most studies were done to assess SP concentrations after administration of analgesics prior to husbandry procedures in calves.

Conclusions: There is a manageable number of studies assessing SP concentrations during painful procedures, conditions, and diseases in cattle. SP seems to be a suitable biomarker for nociception in cattle, but results of research work are heterogeneous, and SP concentrations of calves and adult cattle differ throughout studies. Basic research work is missing and is needed to assess factors other than nociception which might influence the SP concentrations in the blood plasma.

Keywords: Analgesia, Castration, Dehorning, Pain assessment, Pain management, Surgery
As a neurotransmitter (tachykinin), SP is involved in the processing of nocuous sensory information to the brain [13]. SP, which is composed of 11 amino acids (Arg-Pro-Lys-Pro-Gln-Gln-Phe-Phe-Gly-Leu-MetNH₂) [14–16] is synthesized as a propeptide in ribosomes and transported to the nerve ends via axons. Following a (thermal, mechanic, or chemical) noxious stimulus, SP is released from the neurons of the spinal ganglion and can be found in afferent neurons of the dorsal horn of the spinal cord, in cells of the dorsal ganglion, and in the dorsal roots of spinal nerves [16]. SP is primarily released from C-fibers, and its release is described to be slow [17].

SP was first used as a pain marker in bovine medicine in 2008 by [8]. The authors showed that the plasma SP concentrations increased significantly in castrated compared with sham-castrated calves, contrary to the cortisol concentrations, which increased in both groups [8]. Since then, various studies investigating the SP concentrations in adult cattle and calves during different (painful) procedures and conditions have been published. However, SP concentrations vary throughout the literature [8], and high variations between calves have been described [8, 18]. Additionally, Dockweiler et al. (2013) found an age difference in SP concentrations in calves [19]. Also, there are varying reports about the relationship between SP and procedures related to pain such as surgical castration [20] or disbudding [21], with no difference in SP concentrations between control animals and animals which had been treated with analgesic drugs.

Reviews have been published about pain assessment in cattle [22–26], but to this day, there is no systematic review about the use of SP as a biomarker for pain. Therefore, the objective of the present paper was to describe and compare SP concentrations in adult cattle and calves associated with different (painful) procedures, conditions, and diseases. The aim of this review is to be a contribution to the current knowledge by giving an overview of literature concerning research about SP in bovine, and to identify and outline areas of lack of knowledge.

Material and methods

Search strategy and criteria for selection

The present systematic review was done following the study protocol for PRISMA-P (Preferred Reporting Items for Systematic Reviews and Meta-Analysis protocols) as published by Shamseer et al. [27] (Additional file 1) and described by [28]. The literature search was conducted on the 28th of September 2021 and was limited to peer-reviewed articles in English and German. For this systematic review, the following 3 electronic scientific literature databases were used: PubMed (including MEDLINE), Web of Science, and Agricola. The main elements of this review were Cattle, Substance P, and Pain, and the same code was used for all three databases; the population search terms were (cattle OR cow OR calves OR bull OR steer), and the outcome search terms were ("substance P") and (pain* OR nocicept*). For this systematic review, calves were defined as cattle ≤ the age of 12 months.

Selection of studies

According to the search items stated above, studies of all designs and different languages describing the evaluation of Substance P during various procedures in cattle and calves were admitted into the study selection. Studies with English or German titles were included in the search, whereas studies in other languages and studies which were not accessible in any way were omitted. Following the exclusion of all duplicate studies, two authors (TT, MF) independently evaluated all titles of the remaining publications to check if the eligibility criteria (studies about pain assessment in cattle or calves) were fulfilled. Titles including other species than cattle were excluded, as well as reviews. The abstracts of the remaining studies were screened by two authors (TT, MF) for the eligibility criteria, and if a study appeared to be eligible, the full text was retrieved. Full texts were screened by one author (TT) and were included in the systematic review, if the following questions could be answered with yes, as described by [29]:

1) Is the study population either cattle or calves?
2) Is SP used as a biomarker for pain/nociception?
3) Are animals undergoing a painful procedure (such as castration, dehorning, etc.) or condition/disease?
4) Is the article peer-reviewed?

In cases of uncertainty whether a study should be included, the second author (MF) was consulted to decide upon the decision.

Data extraction

The first author (TT) screened all full texts and extracted all data regarding the author, year of publication, number of animals and grouping, timing, and processing of samples, and results (concentrations of SP).

Results

Findings: demographic

The literature search of the three databases resulted in a pool of 236 references; of these, 133 remained after deletion of all duplicate titles. A total of 49 references was excluded after screening of title, resulting in 84 references for screening of the abstract. Out of these references, 48 were excluded due to the reference not
being eligible for the systematic review. Of these, 4 abstracts were not accessible, one was in French and one in Chinese. A PRISMA flow chart presenting an overview of the literature search and study selection is given in Fig. 1, and a publication number diagram is presented in Fig. 2. In total, we included 36 studies into the systematic review. With 69.4% \( (n = 25) \), most studies were conducted on calves, compared with 30.6% \( (n = 11) \) on cattle. Range of publication year was from 2008 to 2021 (Fig. 3), and studies were conducted in the United States of America (USA), Canada, Germany, South America, and the Republic of Korea (Fig. 2). The distribution of painful procedures and conditions/diseases in calves and cattle is given in Table 1.

**Findings: material and methods**

Processing of blood samples \( (n = 36 \) studies) and saliva samples \( (n = 1 \) study) as described in Material and Methods was heterogenous. A summary of inhibitor used to keep SP from degradation, hours until processing and centrifugation of blood samples, matrix (blood plasma or serum) used, temperature at which samples were kept until analysis, method as assaying, and unit used for the evaluation of SP concentrations is presented in Fig. 4. Samples were kept on ice until processing or cooled/refrigerated in 54.1% \( (n = 6) \) and 18.2% \( (n = 2) \) in cattle, and 52% \( (n = 13) \) and 4% \( (n = 1) \) of calves, respectively.

In 27.3% \( (n = 3) \) of studies in cattle, and 44% \( (n = 11) \) studies in calves, there was no information about storing...
of samples for the determination of SP until processing and centrifugation.

**Findings: funding**
Funding information was provided for 84% \((n=21)\) and 81.8% \((n=9)\) studies in calves and cattle, respectively (Additional file 2).

**Findings: calves**
A total of 25 studies evaluating SP concentrations in calves during painful procedures, conditions, or diseases were identified. SP concentrations were evaluated for dehorning \((28\%, n=7)\), castration \((56\%, n=14)\), and other procedures and conditions/diseases \((16\%, n=4)\). Year of publication, authors, grouping of animals (including administration of local anesthesia (LA) prior to painful procedures), time of blood sampling, extractable SP concentrations, and overall results are presented in Table 2.

**Castration**
Most studies using SP to evaluate pain during painful procedures in calves were done using castration as a painful stimulus \((56\%, n=14)\). Study design was heterogeneous (surgical/knife castration: 50% \((n=7)\), band castration: 7.1% \((n=1)\), band and knife castration: 28.6% \((n=4)\), band, cut-and-clamp, and cut-and-pull: 7.1% \((n=1)\), knife castration and branding: 7.1% \((n=1)\)), as was grouping of animals, and findings of the different studies. Coetzee et al. (2008) showed that plasma SP concentrations were significantly \((p=0.042)\) higher in surgical compared with sham-castrated calves [8]. After surgical castration, SP concentration only leveled out after 21 days [31]. According to Meléndez et al. (2017), an overall increase \((p<0.01)\) of SP concentrations was observed after surgical castration [5]. Also, there is an effect of administration of analgesics [36], as well as timing [5], and form of application [37] of NSAIDs on SP concentrations after surgical castration. When comparing surgical, band, and sham castration, there was no effect of treatment on calves of different age groups [32, 33]. Administration of meloxicam resulted in significantly \((p<0.05)\) [10] and by trend \((p=0.06)\) [34] lower SP concentrations in band and surgically castrated compared with control calves. Contrary to that, one reference stated that there was no effect of treatment (band castration, band castration an administration of meloxicam, or sham castration) on SP concentrations in calves [30]. All of the above-mentioned studies were performed without the administration of a LA.
Dehorning
Evaluation of SP concentrations during and after dehorning was described for cautery and scoop dehorning and dehorning with a caustic paste ($n = 4$, $n = 2$, and $n = 1$, respectively). Study design was heterogenous, with variable grouping of animals and treatment with different NSAIDs. Contrary to one study stating that SP concentrations in dehorned calves were significantly ($p = 0.039$) lower in calves treated with meloxicam orally compared to untreated control calves (following a cornual nerve block for both groups) [40], other studies found that neither the administration of oral firocoxib (following a cornual nerve block for both groups) [21], nor carprofen (orally or subcutaneously, following a cornual nerve block for both groups) [42], or topical flunixin meglumine (no administration of LA) [43], had an effect on SP concentrations during or after cautery dehorning. Differences in results were also observed concerning scoop dehorning. Whereas Coetzee et al. (2012) published that an intravenous administration of meloxicam resulted in significantly ($p = 0.038$) lower SP concentrations in calves after scoop dehorning without LA, compared with control calves [39], Glynn et al. (2013) found no differences in SP concentrations during and after scoop dehorning in

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*Fig. 3* Demographic overview of range of publication year and origin of studies (country) in references about Substance P concentrations during painful procedures, conditions, and diseases in adult cattle (dark green) and calves (light green). The largest number of studies was published in 2018, and most studies originated from the United States of America (USA) or Canada.
calves treated with either a placebo, meloxicam, gabapentin, a combination of meloxicam and gabapentin orally, or flunixin meglumine intravenously; all groups received a cornual nerve block prior to dehorning. In the same study, calves which did not receive any systemic analgesia showed significantly \((p = 0.02)\) higher SP concentrations compared with calves treated with a systemic analgesia (137.29 ± 42.97 pg/mL for no analgesia and 63.35 ± 21.25 pg/mL for analgesia, respectively) [41].

No influence of different analgesic regimes of oral meloxicam administration (one or two administrations of meloxicam 24 h apart or placebo treatment on SP concentrations after caustic paste disbudding without LA were published by [44].

Other Studies about the evaluation of SP concentrations during painful procedures or conditions other than dehorning or castration were rare \((n = 4)\). Theurer et al. (2013) investigated the effect of challenging calves with *Mannheimia haemolytica* and found a significant \((p < 0.05)\) interaction between treatment group (challenged compared with control calves) and trial day, with SP concentration being significantly increased in challenged compared with control calves on day 0.5 [48]. Pearson et al. (2019) treated newborn calves following assisted calving with either meloxicam or a placebo and found no differences in SP concentrations between groups over a 24-h period [46]. Studies about painful procedures were published by [18].

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**Table 1** Distribution of painful procedures and conditions in 36 references used to evaluate the Substance P concentrations as a biomarker for pain in the blood plasma and serum of calves and adult cattle. For the present systematic review, calves were defined as cattle \(\leq\) the age of 12 months

| Procedure/Condition          | Total number of articles |
|------------------------------|--------------------------|
| **Calves**                   |                          |
| Castration                   | 14                       |
| Dehorning                    | 7                        |
| Other                        | 4                        |
| **Adult Cattle**             |                          |
| (Induced) Lameness           | 4                        |
| Diseases/Conditions          | 3                        |
| Surgeries/Procedures         | 3                        |
| Other                        | 1                        |
| **Total Number of Articles** | 36                       |
Table 2  Summary of publication year, reference (Ref.), painful procedures/condition/disease, grouping and age of animals, sampling times, Substance P (SP) concentrations and conclusion of 25 studies using SP for the evaluation of nociception in calves. The administration of local anesthesia (LA) is given in the same column as the grouping of animals. In 32% (n = 8), no extractable data was presented.

| Year  | Ref  | Procedure/Disease with/without LA | Grouping | Age/Weight | Sampling Times and Assay Platform | SP Concentrations | Conclusions |
|-------|------|-----------------------------------|----------|------------|------------------------------------|------------------|-------------|
| 2008  | [8]  | Surgical Castration               | - Surgical Castration (n = 5) - Sham Castration (n = 5) - no LA | 4 to 6 months | - Baseline (24 and 12 h before procedure) - immediately after procedure - 10, 20, 30 and 45 min after procedure - 1, 1.5, 2, 2.5, 4 and 4 h after procedure - Competitive Immunoassay | Mean and SEM plasma SP Concentrations Surgical Castration Cmin 303.98 ± 119.73 pg/mL, Cmax 888.92 ± 235.44 pg/mL Sham Castration Cmin 88.68 ± 23.93 pg/mL, Cmax 691.38 ± 71.85 pg/mL | Castrated calves showed significantly (p = 0.042) greater mean plasma SP concentrations for all time points after castration or simulated castration than sham castrated calves. Clear between- and within-calf variations of SP throughout the study period were observed |
| 2013  | [19] | Band castration                   | - CONT (control, n = 20) - BAND (n = 18) - CLAMP (n = 20) - PULL (n = 18) - no LA | 8 weeks (n = 40) | 6 months (n = 40) | - Baseline - 60, 120, 240, 480, and 5760 min after castration - Competitive Immunoassay | No extractable numerical data | SP concentrations differed significantly (p = 0.01) by age; regardless of the method of castration, 6-month-old calves showed higher SP concentrations relative to 8-week-old calves |
|       |      | Cut-and-Clamp                     |                      |            |                                    |                  |             |
|       |      | Cut-and-Pull                      |                      |            |                                    |                  |             |
| 2013  | [30] | Band Castration                   | - BAND (n = 7) - BAND-MEL (n = 7, castration and meloxicam) - SHAM (n = 7, sham castration) - no LA | 300.8 ±4.96 kg | Day 0 - Day 1 - Day 7 of trial - Competitive Immunoassay | LSM plasma SP Concentrations BAND, Day 0 143.05 pg/mL, BAND, Day 1 167.24 pg/mL BAND-MEL, Day 0 158.69 pg/mL, BAND-MEL, Day 1 159.66 pg/mL SHAM, Day 0 166.36 pg/mL, SHAM, Day 1 151.45 pg/mL | There was no difference in plasma SP concentrations across treatments; meloxicam was administered on days -1,0, and 1 in a dose of 10.0, 0.5, and 0.5 mg/kg BW respectively |
|       |      |                                  |                      |            |                                    |                  |             |
| 2014  | [31] | Surgical Castration               | - Flunixine (n = 24) - Placebo (n = 24) - ring block of 2% lidocaine for both groups | 25 ± 2 days | directly before treatment with NSAID/ Placebo - days 1, 2, 3, 7, 14, 21, 28, 35, and 49 - RIA | Mean and SEM plasma SP Concentrations Flunixine 34 ± 1.1 pg/mL, Saline 34 ± 1.1 pg/mL Baseline 41 ± 1.2 pg/mL Day 3 34 ± 1.2 pg/mL Day 21 30 ± 1.2 pg/mL | An effect of day on serum SP concentrations was observed (p < 0.001). SP concentrations were highest at baseline, dropped by day 3, and leveled out by day 21. The application of flunixin meglumine (1.1 mg/kg BW IV) had no effect on the serum SP concentrations; also, there was no interaction between drug and day |
|       |      |                                  |                      |            |                                    |                  |             |
| Year | Ref | Procedure/Disease with/ without LA | Grouping | Age/Weight | Sampling Times and Assay Platform | SP Concentrations | Conclusions |
|------|-----|-----------------------------------|----------|------------|---------------------------------|-------------------|-------------|
| 2016 | [10] | Band Castration Surgical Castration | for both castration methods each  - Meloxicam (*n* = 15)  - Control (*n* = 15)  - no LA | 4 to 5 months | - Day - 2  - 5, 24, 48, and 72 h following castration  - Competitive Enzyme Immunoassay | Mean and SE plasma SP Concentrations  Day - 1  - Band, Meloxicam 243.9 ± 16.4 pg/mL  - Band, Control 268.2 ± 15.6 pg/mL  - Surgical, Meloxicam 249.8 ± 7.8 pg/mL  - Surgical, Control 244.5 ± 11.5 pg/mL  Day 0  - Band, Meloxicam 243.7 ± 13.4 pg/mL  - Band, Control 340.5 ± 23.0 pg/mL  - Surgical, Meloxicam 267.9 ± 11.2 pg/mL  - Surgical, Control 314.7 ± 17.0 pg/mL | Plasma SP concentrations were significantly (*p* < 0.05) higher in control compared with meloxicam (1 mg/kg BW orally) treated animals, both on day 0 and day 1 and both for band as well as surgically castrated calves |
| 2017 | [32] | Band Castration Knife Castration | - CT (sham castration)  - BA (band castration)  - KN (knife castration)  - no LA | 12 calves each per group  - 1 week (*n* = 36)  - 2 months (*n* = 36)  - 4 months (*n* = 36) | - Baseline (D - 1), immediately before castration  - weekly afterwards until end of trial (*w* = sloughing off of testicles of banded calves)  - RIA | SP concentrations  1 week old  - Band Castration 63.9 pg/mL  - Knife Castration 63.9 pg/mL  2 months old  - Control 80.1 pg/mL  - Band Castration 76.2 pg/mL  - Knife Castration 81.0 pg/mL  4 months old  - Control 103.3 pg/mL  - Band Castration 100.3 pg/mL  - Knife Castration 100.0 pg/mL | There was no effect of treatment on SP concentrations in 1-week, 2-months, and 4-months old calves |
| Year | Ref | Procedure/Disease with/ without LA | Grouping | Age/Weight | Sampling Times and Assay Platform | SP Concentrations | Conclusions |
|------|-----|------------------------------------|----------|------------|-----------------------------------|------------------|-------------|
|      | [33] | Band Castration Knife Castration | - CT (sham castration) - BA (band castration) - KN (knife castration) - no LA | 12 calves each per group 1 week ($n = 36$) 2 months ($n = 36$) 4 months ($n = 36$) | - Baseline (D-1) - T0, 60, and 120 Minutes after castration - 7 days (D7) after castration - RIA | LSM serum SP Concentrations Day 0 and 7 after castration 1 week old Control 92.6 pg/mL Band Castration 108.7 pg/mL Knife Castration 100.6 pg/mL 2 months old Control 73.5 pg/mL Band Castration 70.1 pg/mL Knife Castration 66.8 pg/mL 4 months old Control 102.9 pg/mL Band Castration 101.8 pg/mL Knife Castration 102.3 pg/mL 0, 60, 120 min after castration 1 week old Control 72.1 pg/mL Band Castration 69.8 pg/mL Knife Castration 68.0 pg/mL 2 months old Control 72.2 pg/mL Band Castration 70.6 pg/mL Knife Castration 68.9 pg/mL 4 months old Control 103.5 pg/mL Band Castration 101.5 pg/mL Knife Castration 101.1 pg/mL | There was no treatment or interaction effect for SP in 1-week-old calves No treatment differences were seen for SP following castration in 2-months and 4-months-old calves |
|      | [5]  | Knife Castration                  | - 6H (NSAID 6 h prior, $n = 11$) - 3H (NSAID 3 h prior, $n = 12$) - 0H (NSAID 0 h prior, $n = 11$) - no LA | 7 to 8 months | - D-7, D-5, D-2, D-1 before castration - immediately before castration (T0) - 30, 60, 120 and 240 min, after castration - 1, 2, 5, 7, 14, 21, and 28 days after castration - RIA | No extractable numerical data | There was no treatment or interaction effects for SP on the day of castration, but an overall increase in SP concentrations ($p < 0.01$) On days 1 to 28 after castration, there was a treatment x time interaction for SP ($p = 0.01$), with 6H and 3H calves (which received 0.5 mg/kg BW meloxicam SC 6 or 3 h prior to surgery, respectively) showing higher SP concentrations than 0H calves on day 1 after castration; 5 days following castration, SP concentrations tended to be higher in 3H compared with 6H calves |
Table 2 (continued)

| Year | Ref | Procedure/Disease with/without LA | Grouping | Age/Weight | Sampling Times and Assay Platform | SP Concentrations | Conclusions |
|------|-----|-----------------------------------|----------|------------|-----------------------------------|-------------------|-------------|
| 2018 | [30] | Surgical Castration               | CAST + FLU (flunixin meglumine, n = 8); CAST + PLOD (placebo, n = 8); SHAM PLOD (placebo, n = 8); no LA | 9 months | - Baseline on the morning of experiment; 1, 2, 4, 6, 8, 12, 24, 48, and 72 h after treatment application; RIA | No extractable numerical data | Following castration, there was no effect of topical flunixin meglumine (3.33 mg/kg BW) on SP concentrations. Also, there was no time effect or treatment by time interaction between the groups. |
|      |     |                                   |          |            |                                   |                   |             |
|      | [34] | Band Castration Knife Castration  | CT (control, n = 24); BA (band, n = 24); KN (knife, n = 24); and in each group NM (placebo, n = 36); M (Meloxicam, n = 36); no LA | 7 to 8 days | - Day -1; T0, 60, 90, and 120 min after castration; day 1, 2, 3, and 7 after castration; RIA | LSM serum SP Concentrations Minutes after castration Control, NM 91.1 pg/mL; M 93.4 pg/mL; Knife, NM 102.7 pg/mL; Knife, M 99.5 pg/mL; Days after castration Control, NM 52.3 pg/mL; M 99.1 pg/mL; Band, NM 100.2 pg/mL; Band, M 91.7 pg/mL; Knife, NM 99.1 pg/mL; Knife, M 90.1 pg/mL | There was a trend ($p = 0.09$) for SP concentrations to be higher in NM compared with M calves 120 min after castration. Also, SP concentrations were higher ($p = 0.04$) on day 7, and tended to be higher ($p = 0.08$) on day 3 after castration in NM than in M calves. There was also a trend ($p = 0.06$) for SP concentrations to be higher in CT-M, BA-NM, KN-NM calves than BA-M and KN-M calves. |
|      |     |                                   |          |            |                                   |                   |             |
|      | [35] | Knife Castration Branding          | CK (SHAM, n = 23); KN (knife, n = 24); BK (branding and knife, n = 24); and in each group NM (placebo, n = 36); M (Meloxicam, n = 36); no LA | 67 to 87 days | - 24 h (Day -1) before castration; immediately before (T0) castration; 60, 90, 120, 180 min after castration; day 1, 2, 3, and 7 after castration; RIA | LSM serum SP Concentrations Minutes after castration Control 81.8 pg/mL; M 79.4 pg/mL; Branding + Knife, NM 82.6 pg/mL; Branding + Knife, M 700 pg/mL; Days after castration Control 82.2 pg/mL; Knife, NM 78.7 pg/mL; Knife, M 75.8 pg/mL; Branding + Knife, NM 84.5 pg/mL; Branding + Knife, M 814 pg/mL | There was no effect of procedure of medication (Meloxicam, 0.5 mg/kg SC) for SP at any time after the procedure. |
|      |     |                                   |          |            |                                   |                   |             |
|      | [36] | Surgical Castration               | NC-NLF (no castration, no analgesia, n = 10); NC-LF (no castration, analgesia, n = 10); C-NLF (castration, no analgesia, n = 10); C-LF (castration, analgesia, n = 10); ring block with 2% lidocaine hydrochloride for LF treatments | 6.3 ± 0.09 months | - Immediately before castration; 0.5 and 6 h after castration; 1, 3, and 7 days after castration; ELISA | Plasma SP Concentrations 6 h following castration C-NLF 3.09 ng/mL; NC-NLF 0.74 ng/mL; Otherwise, no extractable numerical data | SP concentrations did not differ between groups 30 min after castration; at 6 h after castration, SP concentrations were significantly ($p = 0.003$) higher in C-NLF compared with C-LF (castration with 12 ml lidocaine and 0.5 mg/kg BW flunixin meglumine) (3.09 ng/mL and 0.74 ng/mL respectively). SP concentrations returned to baseline values from day 1 on |
| 2019 | [37] | Knife Castration                  | PO (Meloxicam, 1 mg/kg—BW orally, n = 12); SC (Meloxicam, 0.5 mg/kg—BWSC, n = 11); no LA | 7 to 8 months | - Day -2 and -1 before castration; T0, and 30, 60, 90, 120, 150, and 240 min after castration; day 1, 2, 3, 5, 7, 10, 14, 21, and 29 after castration; RIA | LSM serum SP Concentrations PO 830.0 pg/mL; SC 787 pg/mL | SP concentrations were higher ($p < 0.05$) in PO compared with SC calves. |

**Note:** SP = Somatomedin—C; FLU = flunixin meglumine; PLOD = placebo (Lidocaine 2%); SHAM = sham; LA = local analgesia; CT = control; BA = band; KN = knife; NM = placebo; M = Meloxicam; RIA = radioimmunoassay; PO = orally; SC = subcutaneously.
| Year | Ref | Procedure/Disease with/ without LA | Grouping | Age/Weight | Sampling Times and Assay Platform | SP Concentrations | Conclusions |
|------|-----|----------------------------------|----------|------------|----------------------------------|------------------|-------------|
| 2021 | [38] | Surgical Castration | - SHAM (castration), followed by CAST (24 h later) | 6 weeks (n = 10) | Immediately prior to both procedures (Time 0) - 1, 2, 4, 8, and 12 h after the procedures - Competitive Immunoassay | No extractable numerical data | At later recovery times, SP concentrations were lower in CAST compared with SHAM. Younger calves (6 weeks old) showed lower SP concentrations in CAST than in SHAM (p = 0.00174) |
| | | | - no LA | 3 months (n = 10) | | | |
| | | | | 6 months (n = 10) | | | |
| | | | | | | | |
| Dehorning | | | | | | | |
| 2012 | [39] | Scoop Dehorning | - Meloxicam (n = 6) | 16 to 20 weeks | Baseline (prior to drug or placebo administration) - 5, 10, 15, 29, 30, and 60 min afterwards - 6, 22, 30, 43, and 52 h afterwards - Competitive Immunoassay | Mean and SEM plasma SP concentrations Placebo 114.70 ± 30.84 pg/mL Meloxicam 71.36 ± 20.84 pg/mL | Mean SP concentrations were significantly (p = 0.0038) lower in meloxicam (0.5 mg/kg IV) treated calves compared with control calves. Plasma SP concentrations were estimated to be 0.5 less in the presence than in the absence of meloxicam treatment |
| | | | - Placebo (n = 6) | | | No extractable numerical data | |
| | | | | | | | |
| 2013 | [40] | Cautery Dehorning | - MEL-PRE (NSAID pre-OP, n = 10) | 8 to 10 weeks | - 2 h before procedure (baseline) - 5, 30, 60, 120, 240, 360, and 720 min after dehorning - Competitive Immunoassay | No extractable numerical data | At 120 min after dehorning calves which received meloxicam (1 mg/kg orally) had significantly (p = 0.039) lower SP concentrations than control calves |
| | | | - MEL POST (NSAID post-OP, n = 10) | | | | |
| | | | - CONIF (control, n = 10) | | | | |
| | | | - cornual nerve block with 2% lidocaine hydrochloride for all groups | | | | |
| | | | - MEL + CONIF (n = 8) | | | | |
| | | | - MEL + GABP (gabapentin, n = 6) | | | | |
| | | | - FLU (flunixin meglumine, n = 8) | | | | |
| | | | - cornual nerve block with 2% lidocaine hydrochloride for all groups | | | | |
| | | | | 6 months | - Baseline (-10 min) before dehorning - 5 min after dehorning - 0.5, 1, 2, 4, 6, 8, and 12 h after dehorning - Competitive Immunoassay | Mean and SD plasma SP Concentrations Calves not treated with Analgesia | No differences between treatment groups (Placebo, Meloxicam (1 mg/kg orally), Gabapentin capsules (15 mg/kg orally) or Flunixin meglumine (2.2 mg/kg IV) were found. Mean plasma SP concentrations were significantly (p = 0.002) lower in calves treated with analgesics compared with control calves |
| | | | | | | Calves treated with Analgesia | |
| | | | | | | Gabapentin capsules (15 mg/kg orally, Meloxicam and Gabapentin orally, or Flunixin meglumine (2.2 mg/kg IV) | |
| | | | | | | were found. Mean plasma SP concentrations were significantly (p = 0.002) lower in calves treated with Analgesia compared with control calves |
| | | | | | | compared with control calves | |
| | | | | | | no differences between treatment groups | |
| | | | | | | (Placebo, Meloxicam (1 mg/kg orally), Gabapentin capsules (15 mg/kg orally) or Flunixin meglumine (2.2 mg/kg IV) were found. Mean plasma SP concentrations were significantly (p = 0.002) lower in calves treated with Analgesia compared with control calves |
| | | | | | | compared with control calves | |
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Table 2 (continued)

| Year | Ref  | Procedure/Disease with/ without LA | Grouping                        | Age/Weight | Sampling Times and Assay Platform | SP Concentrations | Conclusions |
|------|------|------------------------------------|---------------------------------|------------|-----------------------------------|-------------------|-------------|
| 2019 | [44] | Dehorning with Caustic Paste       | - M1 (NSAID followed by placebo 24 h later, n = 15) | 3 days     | - Baseline (5 min prior to disbudding) | LSM plasma SP Concentrations | Plasma SP concentrations not different between plasma SP concentrations between CONTROL and M2 (45 mg Meloxicam orally 24 h apart) than SHAM or M1 (45 mg Meloxicam orally, followed by placebo). Calves in SHAM had significantly (p < 0.0001) higher plasma SP concentrations |
|      |      |                                   | - M2 (NSAID twice in 24 h, n = 15) |            | - 24, 48, 72, and 96 h after disbudding | CONTROL 164.47 pg/mL |            |
|      |      |                                   | - CONTROL (n = 16)               |            | - RIA                             | Sham 198.53 pg/mL |            |
|      |      |                                   | - SHAM (sham disbudding, n = 15) |            |                                   | M1 114.50 pg/mL |            |
|      |      |                                   | - no LA                          |            |                                   | M2 144.74 pg/mL |            |
|      |      |                                   |                                  |            |                                   |                   |            |
| 2013 | [45] | Infection with Mannheimia haemolytica | - MH (infected calves, n = 10) | 240.0±13.1 kg | - Before challenge (D0) | No extractable numerical data | There was a significant (p < 0.05) interaction between treatment group and trial day for SP concentrations. SP concentrations were significantly increased in MH compared with CT calves at D0 and decreased to average concentrations on D7 |
|      |      |                                   | - CN (control, n =8)             |            | - 12 h after inoculation           |                   |            |
|      |      |                                   | - no LA                          |            | - day 1, 2, 3, 7, and 9 after inoculation |                   |            |
|      |      |                                   |                                  |            | - Immunoassay Kit (ELISA)         |                   |            |
| 2018 | [18] | Umbilical Surgery                  | - CON (meloxicam treated calves, n = 10) | 37±8 days  | - Baseline (-1 h before surgery) | Median plasma SP Concentrations | Plasma SP concentrations were lower in MET (40 mg/kg BW metamizole IV and 0.5 mg/kg BW meloxicam IV) compared with CON (0.5 mg/kg BW meloxicam IV) at all times during and after surgery. In CON, plasma SP concentrations were significantly different from baselines at T5 (p = 0.027), T30 (p = 0.006) and T90 (p = 0.02). Calves of CON did not reach baseline plasma SP concentrations during the trial period |
|      |      |                                   | - MET (metamizole and meloxicam treated calves, n = 11) |            | - 5, 15, 30, 45, 90, 150, and 510 min after start of surgery (skin incision) | Baseline |            |
|      |      |                                   | - no LA                          |            | - ELISA                           | T+ 5 986.8 pg/mL |            |
|      |      |                                   |                                  |            |                                   | T+ 30 1217.2 pg/mL |            |
|      |      |                                   |                                  |            |                                   | MET T+ 6 541.1 pg/mL |            |
|      |      |                                   |                                  |            |                                   | T+ 150 555.6 pg/mL |            |
|      |      |                                   |                                  |            |                                   | T+ 510 547.5 pg/mL |            |
| 2019 | [46] | Assisted Calving                  | - Meloxicam (n = 17)             | Newborn    | - Birth (within 10 min of delivery) | No extractable numerical data | There was no significant difference between placebo and meloxicam (0.5 mg/kg BW SC) treated calves for SP concentrations over the 24-h period |
|      |      |                                   | - Placebo (n = 16)               |            | - 1, 4, 24 h and 7 and 10 days after delivery | - RIA |            |
|      |      |                                   |                                  |            |                                   |                   |            |
| 2020 | [47] | Tail Docking                      | - A (Amputation, n = 8)          | 8 to 10 weeks | - D-2 to Day 2 at 11:10 am daily | No extractable numerical data | There was a trend (p = 0.007) for a difference between A and K for the mean SP concentrations. After differentiation of values after tail docking and handling, there was no difference in SP concentrations between groups |
|      |      |                                   | - K (Control, n =8)              |            | - D0 at 08 10 AM prior to tail docking via rubber ring | - ELISA |            |

**BW** Bodyweight, **IV** Intravenously, **OP** Surgery, **SC** Subcutaneously, **RIA** Radioimmunoassay, **ELISA** Enzyme Linked Immunosorbent Essay
and [47]. Tschoner et al. (2018) investigated the effect of different analgesic treatments (either meloxicam and a placebo, or meloxicam and metamizole) prior to surgery to correct umbilical hernia under isoflurane anesthesia without LA in calves. Animals treated with both analgesics showed lower SP concentrations during and after umbilical surgery, compared with animals treated with only one analgesic [18]. Another study showed that tail amputation with a rubber band did not have an effect on SP concentration in calves [47].

Findings: adult cattle
A total of 11 studies evaluating SP concentrations in adult cattle during painful procedures, conditions, or diseases were identified. SP concentrations were evaluated for lameness (36.4%, n = 4), diseases (27.3%, n = 3), surgeries (27.3%, n = 3), and other procedures (9.1%, n = 1). Year of publication, authors, grouping of animals, time of blood sampling, extractable SP concentrations, and overall results are presented in Table 3.

Lameness
Three studies investigated SP concentrations in adult cattle after experimentally induced lameness (either with Oligofructose, n = 1 [49], or Amphotericin B, n = 2 [50, 52]). In each of these three studies, grouping and treatment of animals was heterogenous. Bustamante et al. (2015) showed that mean plasma SP concentrations increased significantly (p < 0.05) 6 h after induction of lameness with oligofructose, with a peak 12 h after the lameness induction (2.20 ± 0.47 mg/mL). Significant differences (p < 0.001) were found at each time point after baseline sampling between control and treatment group [49]. Kleinhenz et al. (2019) and Warren et al. (2021) investigated the effect of different NSAIDs on SP concentrations in cattle with Amphotericin B induced lameness and both stated that there were no significant differences in SP concentrations between animals treated with either a NSAID or a placebo [50, 52].

Only one study compared SP concentrations in a population of cattle with different mobility scores (MS, MS 0 being not lame, to MS 3 being severely lame) and stated that mean SP concentrations increased linearly with the mobility score. Animals with a MS 3 showed significantly (p = 0.000043) higher SP concentrations compared with MS 0 (0.61 ± 0.12 ng/mL and 0.25 ± 0.09 ng/mL, respectively) [51].

Diseases and conditions
Studies describing SP concentrations during painful conditions and diseases were limited to clinical Metritis (n = 1), parturition (n = 1), and uterine torsion (n = 1). Out of these, two studies were part of one larger trial [53, 55]. In 2018, Barragan et al. (2018) compared circulation SP concentrations of cows with or without clinical metritis (diagnosed on day 7 ± 3 after parturition). Cows with clinical metritis had significantly (p < 0.01) higher circulation SP concentrations compared with sound animals (41.15 ± 5.38 pg/mL and 37.73 ± 5.41 pg/mL, respectively) [53]. In a follow up paper, the authors found no difference in circulation SP concentrations between animals treated with 100 mg/kg acetylsalicylic acid at a 12-h interval for four times after parturition, compared with animals treated with a placebo. The SP concentrations increased, with a peak at 168 h after parturition. Cows suffering from dystocia had significantly (p < 0.01) higher SP concentrations at 168 ± 72 h compared with cows with eutocia; also, primiparous cows showed significantly (p = 0.04) higher circulation SP concentrations than multiparous cows [55].

Regarding uterine torsion, serum SP concentrations were significantly (p < 0.01) higher in cows during parturition compared with cows with uterine torsion (49.6 ± 14.5 pg/mL and 32.8 ± 14.1 pg/mL). Healthy control cows had significantly (p < 0.01) lower SP concentrations than intrapartum cows (37.9 ± 10.5 pg/mL and 49.6 ± 14.5 pg/mL, respectively) [54].

Surgeries
SP as a biomarker for pain during surgeries has not been used extensively in adult cattle. Whitlock et al. (2012) evaluated SP concentrations following electroejaculation and found that mean plasma SP concentrations was not different between control (93 ± 17.2 pg/mL), probed (79.1 ± 17.2 pg/mL) and electroejaculated (77.2 ± 17.2 pg/mL) bulls [56]. Another study showed that mean plasma SP concentrations did not differ between female cattle either subjected to ovariectomy following administration of butorphanol, xylazine, and ketamine, ovariectomy without the administration of any analgesia, or palpation only (78.6 pg/mL, 79.8 pg/mL, and 78.7 pg/mL, respectively). Tschoner et al. (2020) investigated the effect of an administration of 0.02 mg/kg BW xylazine or the equivalent amount of 0.9% saline intravenously before laparoscopic abomasopexy following local and systemic analgesia on SP concentrations in cattle and found no differences during and after the surgery between both groups [58].

Other
One study described the effect of long-distance transporting (16 h, approximately 1.316 km) on plasma SP concentrations in beef steers, and the effect of the administration of a NSAID (meloxicam) on plasma SP concentrations. The plasma SP concentrations increased significantly (p < 0.0026) after transportation, but there
Table 3  Summary of publication year, reference (Ref.), painful procedures/condition/disease, grouping and age of animals, sampling times, Substance P (SP) concentrations and conclusion of 11 studies using SP for the evaluation of nociception in adult cattle. The administration of local anesthesia (LA) is given in the same column as the grouping of animals. Data was not extractable from 9.1% (n = 1) of papers.

| Year | Ref Procedure/Disease with/without LA | Grouping | Weight/Age/Lactation | Sampling Times and Assay Platform | Substance P Concentrations | Conclusion |
|------|-------------------------------------|----------|----------------------|----------------------------------|---------------------------|------------|
| 2015 | [49] Lameness Oligofructose induced-lameness | Treatment (13 g/kg BW oligofructose orally, n = 6) - Control (n = 6) - no LA | 250 to 300 kg | 48 and 24 h before induction of lameness - 6, 12, 24, 36, and 48 h after induction of lameness - ELISA | Mean plasma SP concentrations Control 0.26 to 0.42 ng/mL 12 h after lameness induction. Treatment group 2.20 ± 0.47 ng/mL | Mean plasma SP concentrations increased significantly (p < 0.05) 6 h after lameness was induced (treatment group), with a peak 12 h, and decreasing after 48 h after induction. Plasma SP concentrations differed significantly (p < 0.001) at every time point after baseline between treatment and control group |
| 2019 | [50] Ampothericin B induced-lameness | - L + F (lameness + flunixin, n = 10) - L + P (lameness + placebo, n = 10) - S + P (sham + placebo, n = 10) - no LA | 2nd or 3rd lactation | 6 h before induction of lameness - 1, 2, 8, 24, 48, 72, 96, and 120 h after lameness induction - RIA | Mean SP concentrations L + F 84.59 pg/mL; 95% CI: 73.12 to 96.05 pg/mL L + P 81.89 pg/mL; 95% CI: 72.16 to 91.62 pg/mL S + P 70.59 pg/mL; 95% CI: 55.72 to 85.46 pg/mL | The L + P group had similar SP concentrations to the L + F (topical flunixin meglumine (3.33 mg/kg) for 3 days every 24 h) and S + P group |
| 2020 | [51] Lameness | - MS 0 (n = 25) - MS 1 (n = 25) - MS 2 (n = 25) - MS 3 (n = 25) (on the basis of mobility scoring (MS)) - no LA | 1st to 6th lactation, 400 to 500 kg | 1 sample at last follow up visit - ELISA | Mean SP Concentrations MS 0 0.25 ± 0.09 ng/mL MS 1 0.21 ± 0.13 ng/mL MS 2 0.42 ± 0.12 ng/mL MS 3 0.61 ± 0.12 ng/mL | The mean SP concentrations increased linearly with the increase of MS score. Animals in M3 showed a significant (p = 0.000043) increase in SP concentrations compared with MS 0 animals |
| 2020 | [52] Ampothericin B induced-lameness | - LAME + FLU (flunixin, n = 12) - LAME + MEL (meloxicam, n = 12) - LAME + PLBO (placebo, n = 12) - SHAM + PLBO (not lame and placebo, n = 12) - no LA | 24 h before induction of lameness - 0, 2, 8, 24, 48, 72, 96 and 120 h after induction of lameness - RIA | Log LSM SP concentrations LAME + MEL 203 pg/mL (95% CI: 1.93, 2.14 pg/mL) LAME + FLU 200 pg/mL (95% CI: 1.90, 2.11 pg/mL) LAME + PLBO 198 pg/mL (95% CI: 1.88, 2.09 pg/mL) SHAM + PLBO 207 pg/mL (95% CI: 1.97, 2.17 pg/mL) | There were no differences between treatments flunixin meglumine at 2.2 mg/kg BW IV, Meloxicam at 1 mg/kg BW orally, or a placebo 2 × every 24 h or over time for any of the investigated time points |
### Table 3 (continued)

| Year | Ref | Procedure/Disease with/without LA | Grouping | Weight/Age/Lactation | Sampling Times and Assay Platform | SP Concentrations | Conclusions |
|------|-----|-----------------------------------|----------|---------------------|-----------------------------------|------------------|-------------|
| **Diseases** | | | | | | | |
| **2018** | [53] | Clinical Metritis | - CM (Clinical metritis, \(n = 70\))<br>- NO-CM (no clinical metritis, \(n = 88\))<br>- no LA | - Day 1<br>- RIA | Circulating SP Concentrations<br>CM cows \(41.15 \pm 5.38\) pg/mL<br>NO-CM cows \(37.73 \pm 5.41\) pg/mL | The SP concentrations were significantly \((p = 0.01)\) higher in CM compared with NO-CM cows |
| **Intrapartum Uterine Torsion** | [54] | - Intrapartum uterine torsion \((n = 20)\)<br>- Healthy controls \((n = 36)\)<br>- Intrapartum without uterine torsion \((n = 15)\)<br>- no LA | - Day 1<br>- ELISA | Serum SP concentrations<br>Control \(37.9 \pm 10.5\) pg/mL<br>Intrapartum (no uterine torsion) \(49.6 \pm 14.5\) pg/mL<br>Intrapartum (uterine torsion) \(32.8 \pm 14.1\) pg/mL | There was no difference in circulating SP concentrations between both treatments. SP concentrations increased after parturition with the highest levels at 168 h. An interaction \((p = 0.07)\) between calving ease and hour after calving was observed; DYS cows showed higher concentrations of SP at 168 \(\pm 72\) h compared with EUT \((p = 0.02)\), and PRIM cows showed higher circulating SP concentrations compared with MULT cows \((p = 0.04)\). There was no difference in SP concentrations between animals with a different number of clinical disease events |
| **2020** | [55] | Parturition | PRIM (primiparous, \(n = 47\))<br>and MULT (multiparous, \(n = 105\)), also EUT (eutocia) and DYS (dystocia) divided into the following treatment groups:<br>- ASP (acetylsalicylic acid, \(n = 76\), including 38 DYS and 38 EUT)<br>- PLC (placebo, \(n = 76\), including 38 DYS and 38 EUT) categorized as<br>- NO-EVT (no disease)<br>- SI-EVT (single disease)<br>- MU-EVT (multiple diseases)<br>- no LA | - 12, 24, 36, and 48 h before parturition (before each treatment administration 4 consecutive treatments at 12 h interval with either acetylsalicylic acid \(1.00\) mg/kg orally) or a placebo)—168 \(\pm 72\) h after parturition<br>- RIA | Circulating SP Concentrations<br>ASP \(56.76\) pg/mL, 95% CI: 55.16–58.41<br>PLC \(55.95\) pg/mL, 95% CI: 54.36–57.57<br>At 168 \(\pm 72\) h after parturition<br>DYS \(64.99\) pg/mL, 95% CI: 62.08–68.06<br>EUT \(60.33\) pg/mL, 95% CI: 57.65–63.15<br>PRIM \(57.62\) pg/mL, 95% CI: 55.62–59.68<br>MULT \(55.11\) pg/mL, 95% CI: 53.83–56.42 | There was no difference in circulating SP concentrations between both treatments. SP concentrations increased after parturition with the highest levels at 168 h. An interaction \((p = 0.07)\) between calving ease and hour after calving was observed; DYS cows showed higher concentrations of SP at 168 \(\pm 72\) compared with EUT \((p = 0.02)\), and PRIM cows showed higher circulating SP concentrations compared with MULT cows \((p = 0.04)\). There was no difference in SP concentrations between animals with a different number of clinical disease events |
### Table 3 (continued)

| Year | Ref | Procedure/Disease with/without LA | Grouping | Weight/Age/Lactation | Sampling in Times and Assay Platform | SP Concentrations | Conclusions |
|------|-----|-----------------------------------|----------|----------------------|-------------------------------------|--------------------|-------------|
| **Surgeries** | | | | | | | |
| 2012 | [56] | Electroejaculation | - EEJ<br>- Probed, no EEJ<br>- Control<br>\(n = 9\), each bull receiving each treatment<br>- no LA | 14.15 ± 0.14 months, 501.9 ± 14.3 kg | - 60 and 30 min before treatment<br>- 0 min and immediately after treatment<br>- 10, 20, 30, 45, 60, 75, 90, and 120 min after treatment<br>- ELISA | MEAN and SEM plasma SP Concentrations<br>Control Bulls 93.4 ± 17.2 pg/mL<br>Probed Bulls 79.1 ± 17.2 pg/mL<br>Bulls after Electroejaculation 77.2 ± 17.2 pg/mL | Mean plasma SP concentrations were not different between groups. An effect of time (\(p < 0.0001\)) could be observed, but no effect of treatment. Also, there was no interaction of treatment and time on SP concentrations |
| 2020 | [57] | Ovariectomy | - PALP (sham procedure, \(n = 14\))<br>- SPAY (ovariectomy, \(n = 15\))<br>- BXKM (spay + NSAID, \(n = 15\); Combination of butorphanol (0.01 mg/kg BW), xylazine (0.02 mg/kg BW), and ketamine (0.04 mg/kg BW) IM 5 min pre OP and oral meloxicam (1 mg/kg) immediately before surgery<br>- no LA | 322 ± 27.0 kg | - D-1<br>- D0 (at time of procedure)<br>- 1, 2, and 4 h after procedure<br>- Day 1, 2, 4, and 7 after procedure<br>- Competitive Immunoassay | LSM plasma SP Concentrations<br>PALP 78.7 pg/mL<br>SPAY 79.8 pg/mL<br>BXKM 78.6 pg/mL | Regarding SP concentrations, there was no treatment or treatment x interaction effect between groups |
| [58] | | Endoscopic Abomasopey | - CON (placebo, \(n = 14\))<br>- XYL (xylazine, \(n = 14\))<br>- local infiltration of skin with 2% procain hydrochloride for both groups | 60 ± 2.0 years, 662.3 ± 110.7 kg | -180 min (Baseline) before surgery<br>at the start of surgery<br>-15, 30, 45, 60, 90, 120, and 180 min after start of the surgery<br>-24 h after the start of the surgery<br>- ELISA | Mean plasma SP Concentrations<br>Baseline Values<br>CON 555.37 ± 252.77 pg/mL<br>XYL 490.60 ± 219.62 pg/mL | There was no significant difference between plasma SP concentrations between CON and XYL (0.02 mg xylazine IV 15 min before the start of the surgery) at any time point |
| **Other** | | | | | | | |
| 2014 | [59] | Long distance transportation | - MEL (meloxicam)<br>- PLACEBO<br>- no LA | 15 to 17 months, 201 to 465 kg | Baseline at Time 0, immediately before treatment and 24 and 144 h after baseline sampling<br>- RIA | No extractable numerical data | SP concentrations increased significantly (\(p < 0.0026\)) after transportation. There was no treatment, or treatment x time interaction, as well as no association between MEL (Meloxicam, at 1 mg/kg BW orally) and SP |

\(BW\) Bodyweight, \(IV\) Intravenously, \(OP\) Surgery, \(SC\) Subcutaneously, \(RIA\) Radioimmunoassay, \(ELISA\) Enzyme Linked Immunosorbent Essay
was no effect of treatment with meloxicam on the SP concentrations [59].

Discussion

Findings of the systematic review

The objective of the present study was to give an overview of SP concentrations during and after painful procedures and conditions in calves and cattle. Our aim was to present the different SP concentrations evaluated in the blood plasma for surgeries, procedures, conditions, and diseases, and perform a meta-analysis, if possible. Additionally, we wanted to quantify the existing body of research, also highlighting potential areas where knowledge could and should be increased.

The manageable number of articles extracted from the data bases (n = 236) and the small number of references which could be included in this systematic review (n = 36) provides evidence that research about SP to evaluate pain in cattle is rare. Even with the number of 36 references, none could be included into a meta-analysis, as study design, grouping, and procedures were too heterogenous. Only a small number of studies compared painful conditions with sham or no intervention control groups, such as [8] for castration and [47] for tail docking in calves, or [51] for lameness in cattle. Most studies used different biomarkers for pain, including SP, to evaluate the effect of different analgesic regimes and the different routes of application (oral, intravenously, subcutaneous) of these analgesics on painful surgeries and procedures. Therefore, the evaluation of SP was not the main focus of these studies, and basic research work is missing. Another problem was the style in which p-values were presented; not all papers presented p-values as accurate numbers, which might be related to the guidelines of the different publishing journals.

Results of studies were heterogenous, especially for dehorning procedures. Whereas Allen et al. (2013) showed that the administration of meloxicam results in significantly lower SP concentrations after cauter dehorning [40], other studies found no effect of systemic analgesics on SP concentrations after different methods of dehorning [21, 42, 43]. Some authors [21, 40, 42] used cornual nerve blocks for local anesthesia of the tissue, whereas some [43] did not work with any local anesthesia. Studies have shown that pre-emptive analgesia prevents the onset of nociception [60, 61]. The administration of multimodal pain management (which is a combination of sedatives, local anesthesia, and nonsteroidal anti-inflammatory drugs) is recommended prior to a painful procedure [61–63]. Different combinations of sedatives, and/or local anesthetics and nonsteroidal anti-inflammatory drugs throughout the studies could explain the inconsistency of SP concentrations in these studies. However, other factors need to be considered, as some studies found no difference in SP concentrations in animals only treated with systemic and no local anesthesia [20, 43].

To this day, no studies describing the SP concentrations in healthy, untreated, and not stressed adult cattle or calves have been described to evaluate physiological ranges of SP concentrations in cattle – although studies showed that SP concentrations differed significantly by age, with 6-months-old calves showing higher concentrations than 8-week-old calves [19]. As there was no consistency among age groups of animals included in studies, SP concentrations cannot be compared easily. Also, gender seems to have an influence on SP; Stock et al. (2016) observed that that SP concentrations were higher in female compared with male calves [42]. In calves, male and female animals were used for the different studies, which, again, makes comparison of concentrations difficult. Even within the same gender and age group, high between- and within-calf variations were found [8]. SP also seems to vary depending on temperament. Kasimanickam et al. (2019) showed that SP concentrations were significantly (p < 0.05) higher in excitable compared with calm female cattle prior to weaning and at breeding [64].

Another problem with the present data was that not all references offered numerical data. Some studies only presented graphical data [40], some studies none at all [47]. However, even if data could be extracted, processing of samples for the determination of SP was different throughout the studies, making a comparison of SP concentrations hard. Previous research showed that the temperature blood samples are kept at until further processing, and use of different enzyme inhibitors influence the SP concentrations in the blood plasma [65]. Numerous biological processes can affect the SP concentrations in blood samples after harvesting of blood; therefore, samples should be processed with the same time between collection and harvesting for all samples, and kept on ice until further processing [65]. As this information is not given in all references, and vary throughout the studies, SP concentrations may have been affected by this.

In human medicine, extensive research about SP has been done [13, 14, 16]. The first study describing SP in cattle included in this systematic review dates from 2008. Studies concentrating on pain research in cattle have been neglected for a long period of time, compared with pain research in companion animals and horses – only in the last years did researchers focus their work on pain and pain management in cattle [3, 66]. Pain scoring systems for cattle have been established [6, 67] and the public concern with the welfare of dairy and beef cattle has been raised [68]. The increased interest in pain management in cattle might have resulted in the search for a new and objective biomarker for pain, such as SP.
The largest number of studies about SP was published in 2018. However, even if SP is a promising tool to differentiate between stress (caused by e.g. handling) and distress associated with nociception [8], SP has not yet achieved the status of an objective biomarker for nociception which can be used exclusively and without other parameters. Until now, it is recommended to assay SP in combination with cortisol to identify if SP is released due to nociception or stress [44]. Nearly all the references included in this systematic review do not use SP exclusively for the evaluation of nociception, but in combination with other subjective [47, 58] or objective [43, 49, 69] parameters. Other disadvantages of the use of SP have been reviewed recently [25], and include the limited use in the field practice due to the necessary processing of the samples after harvesting of the blood, analysis which can only be done with ELISA [47, 49, 58] or radio immunoassay kits [20, 31, 59], and high costs for the analysis with ELISA kits, e.g. 398.00 Euros/96 wells (Enzo, Enzo Life Sciences GmbH, Lörrach, Germany [70]). Also, the varying study results, as well as the high between- and within-calf variations [8] of SP concentrations might limit the use of SP as an everyday tool for pain assessment.

The largest number of studies was conducted on the effect of different analgesics on dehorning and castration in calves – these are common husbandry procedures, especially in the USA [24, 39], where most studies was performed. In the USA, no drugs are federally approved for pain mitigation during these procedures [71], and they are often performed without the use of analgesia [72, 73]. As castration and dehorning are necessary due to e.g. facility design and provision of human safety, and minimizing the pain the animals are experiencing is important [71], the high number of studies concentrating on the effects of analgesics during castration or dehorning can be explained. Also, a recent survey about the attitudes of veterinarians and producers regarding the use of analgesia in beef cattle showed that analgesia was used more frequently in cattle with increased age, regardless of the procedure or disease, and most frequently or always for abdominal surgery, dehorning, lameness, or pneumonia, regardless of the age of the animal [74].

Studies evaluating the effect of different analgesics on animals undergoing painful procedures are necessary, and veterinarians benefit from these studies by receiving guidelines how to improve animal welfare. However, little work has been done in the area of basic research work about the suitability of SP as a biomarker for pain in cattle so far. Studies in human medicine showed that SP plays a role in the activation of the immune system, chemotaxis of granulocytes, and migration of cells to the location of inflamed tissue [16, 75]. SP concentrations increase during an inflammatory process [16, 75, 76]. The same can be said for conditions of emotional stress [77]. In cases of depressions and states of anxiety, the neurotransmission of SP is impaired [78, 79]. Therefore, states of stress and inflammation in cattle could influence the SP concentrations in the blood plasma; however, no explicit research work to answer these questions has been done to this day, which is one of the great limitations of using SP as a biomarker for pain in cattle. Also, no basic values or reference ranges have been established yet, which somehow complicated the comparison of SP concentrations evaluated in different studies.

**Methodology and limitations**

This systematic review was done following the PRISMA guidelines [27] to reduce the possible risk of bias due to the analysis and the study selection process. As the exact study type has not been determined when the research work for this systematic review was started, and it was unclear whether a meta-analysis could be performed, a pre-specified protocol of this systematic review was not registered, as has been described for other systematic reviews [28]. Also, registration via PROSPERO is only possible for systematic reviews conducted in human medicine/research. To assure a systematic review process, the authors defined an agenda which was followed to select the studies included in this systematic review. Titles and abstracts were screened independently by two authors to reduce the risk of bias, and full-text screening was done following previously specified guidelines. To assess if data were eligible for a meta-analysis, data were discussed with a statistician as described [28].

**Risk of bias**

We used three search engines, to try to not miss any relevant papers or references; using more than one search engine should have reduced the possibility of missing papers. As titles and abstracts were included in the search for keywords, it is unlikely that a large number of papers was not found. Apart from 4 abstracts not being accessible, and 2 abstracts being excluded due to not being in English, all papers which were included in the full-text screening could be assessed. Omitting studies due to language barriers can negatively impact the outcome of a systematic review. However, for this review only two studies could not be included due to this reason – therefore, a bias through limited access should be excluded.

The references we included in this systematic review originate predominantly from the USA and Canada, with only a few studies from other countries. As we evaluated a laboratory parameter, and analysis was done similarly in most studies, the studies included
in this systematic review should be representative for other countries as well.

In nearly all the references, funding information was provided, either for the study itself or the authors positions. As published results included studies observing both a positive [10, 39, 40] as well as no [20, 42, 43] effect of NSAIDs on SP concentrations during different procedures, a publication bias due to the influence of the source funding the studies seems unlikely – especially as funding mostly came from animal welfare organizations, national research councils, or universities.

Conclusion
Pain in cattle is a major welfare problem, and the need for objective parameters to assess pain is evident. Our work shows that results of research work about SP as a pain biomarker in cattle is heterogenous, and concentrations differ throughout studies and study designs. Basic research work is needed to evaluate if SP concentrations are largely influenced by nociception, or also by stress and states of inflammation. Also, reference ranges should be established to make comparison of concentrations between sound animals, and animals in pain, easier. Therefore, this systematic review should aid researchers with their decision on objectives and study design for future research. Future studies on the suitability of SP as a biomarker for pain in cattle can improve the pain management and welfare of adult cattle and calves.

Abbreviations
LA: Local anesthesia; NSAID: Non-steroidal anti-inflammatory drug; PRIMA: Preferred Reporting Items for Systematic Reviews and Meta-Analysis; SP: Substance P; USA: United States of America.

Supplementary Information
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Additional file 1. PRISMA-P checklist for the systematic review “Substance P concentrations in adult cattle and calves during different painful procedures and conditions – a systematic review” according to Shamseer et al. (2015).

Additional file 2. Funding information for 36 references (Ref) included in the systematic review “Substance P concentrations in adult cattle and calves during different painful procedures and conditions – a systematic review” if no funding information was retrievable, this is indicated as “none given”.

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Authors’ contribution
TT conceived and designed the study. TT and MF were involved in the screening of the articles for eligibility. TT screened the full-text articles, extracted the data, and wrote the manuscript. MF assisted with the writing of the manuscript and reviewed the manuscript. All authors approved of the submission. All authors read and approved the final manuscript.

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Availability of data and materials
The data used in this work is indicated and lies with the author. It can be assessed via the corresponding author.

Declarations
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Not applicable.

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Competing interests
The authors declare that they have no competing interests.

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References
1. Huxley JN, Whay HR. Current attitudes of cattle practitioners to pain and the use of analgesics in cattle. Vet Rec. 2006;159(20):662–8.
2. Remnant JG, Tremlett A, Huxley JN, Hudson CD. Clinical attitudes to pain and use of analgesia in cattle – Where are we 10-years on? Vet Rec. 2017;181(15):400.
3. Hudson C, Whay H, Huxley J. Recognition and management of pain in cattle. In: Prac. 2008;30(3):126–34.
4. Fraser AF, Broom DM. Describing, recording and measuring behaviour. In: Fraser AF, editor. Farm animal behaviour and welfare. 3rd ed. Broom DM: CAB International; 1990. p. 7–16.
5. Meléndez DM, Martí S, Pajor EA, Moya D, Gellaty D, Janzen ED, Schwartzkopf-Genswein KS. Effect of timing of subcutaneous meloxicam administration on indicators of pain after knife castration of weaned calves. J Anim Sci. 2017;95(12):S218–29.
6. Gleerup KB, Andersen PH, Munksgaard L, Forkman B. Pain evaluation in dairy cattle. Appl Anim Behav Sci. 2015;171:25–32.
7. Molony V, Kent JE, Robertson IS. Assessment of acute and chronic pain using different methods of castration in calves. Appl Anim Behav Sci. 1995;46(1):33–48.
8. Coetzez JE, Lubbers BV, Toerber SE, Gehring R, Thomson DU, White BJ, Apley MD. Plasma concentrations of substance P and cortisol in beef calves after castration or simulated castration. Appl Anim Behav Sci. 2008;69(6):751–62.
9. Heinrich A, Duffield TF, Lissimore KD, Millman ST. The effect of meloxicam on behavior and pain sensitivity of dairy calves following cauterization and dehorning with a local anesthetic. J Dairy Sci. 2010;93(6):2450–7.
10. Olson ME, Ralston B, Burwash L, Matheson-Bird H, Allan ND. Efficacy of oral meloxicam suspension for prevention of pain and inflammation following band and surgical castration in calves. BMC Vet Res. 2016;12(1):102.
11. Coetzez JE, Lechtermberg KF, Stock ML, Kukanich B. Pharmacokinetics and effect of intravenous nalbuphine in weaned Holstein calves after surgical castration. J Vet Pharmacol Ther. 2014;37(2):169–77.
12. Stewart M, Stafford KJ, Dowling SK, Schaefer AL, Webster JR. Eye temperature and heart rate variability of calves disbudded with or without local anaesthetic. Physiol Behav. 2008;90(4–5):769–72.
13. DeVane L. Substance P: a new era, a new role. Pharmacotherapy. 2001;21(9):1061–9.
14. Rupniak NMJ, Kramer MS. Discovery of the antidepressant and anti-emetic efficacy of substance P receptor (NK 1) antagonists. Trends Pharmacol Sci. 1999;20(12):485–90.
of pain, inflammation, and stress in lactating dairy cows diagnosed with clinical metritis. J Dairy Sci. 2018;101(9):8248–58.

54. Sickinger M, Roth J, Failing K, Wehrend A. Serum neuropeptide concentrations in cows with intrapartum uterine torsion. Anim Reprod Sci. 2018;196:195–4.

55. Barragan AA, Bauman LM, Schuenemann GM, Velez J, Lakritz J, Coetzee JF, Gonzalez JDR, Píeiro JM, Menichetti B, Bas S. Administration of acetylsalicylic acid after parturition in lactating dairy cows under certified organic management: Part II. Biomarkers of nociception, inflammation, and stress. J Dairy Sci. 2020;103(11):1713–22.

56. Whitlock BK, Coffman EA, Coetzee JF, Daniel JA. Electroejaculation increased vocalization and plasma concentrations of cortisol and progesterone, but not substance P, in beef bulls. Theriogenology. 2012;78(4):737–46.

57. Lauder JK, Marti S, Schwartzkopf-Genswein KS, Jelinski MD, Janzen ED. Measuring behavioral and physiological responses to pain mitigation for ovariectomy in Bos taurus yearling beef heifers. J Anim Sci. 2020;98(1):skz386.

58. Tschoner T, Zablotski Y, Knubben-Schweizer G, Feist M. Effect of xylazine administration before laparoscopic abomasopexy to correct left displaced abomasum on markers of stress in dairy cows. J Dairy Sci. 2020;103(10):9318–31.

59. Van Engen NK, Stock ML, Engkelien T, Vann RC, Wulf LW, Karriker LA, Busby WD, Lakritz J, Carpenter AJ, Bradford BJ, et al. Impact of oral meloxicam on circulation physiological biomarkers of stress and inflammation in beef steers after long distance transportation. J Anim Sci. 2014;92(2):498–510.

60. Otto K. S. Schmerztherapie. In: Otto K, editor. Schmerztherapie bei Klein-, Heim- und Versuchstieren. Berlin: Parey; 2001. p. 51–87.

61. Anderson DE, Muir WW. Pain management in cattle. Vet Clin North Am Food Anim. 2005;21(3):623–35.

62. Faulkner PM, Weary DM. Reducing pain after dehorning in dairy calves. J Dairy Sci. 2000;83(9):2037–41.

63. Anderson DE, Edmondson MA. Prevention and management of surgical pain in cattle. Vet Clin North Am Food Anim. 2013;29(1):157–84.

64. Kasimanickam RK, Hall JB, Estill CT, Kastelic JP, Joseph C, Abdel Aziz RL, Nak D. Flunixin meglumine improves pregnancy rate in embryo recipient beef cows with an excitable temperament. Theriogenology. 2018;107:70–7.

65. Mosher RA, Coetzee JF, Allen PS, Havel JA, Griffith GR, Wang C. Effects of sample handling methods on substance P concentrations and immuno-reactivity in bovine blood samples. Am J Vet Res. 2014;75(5):1099–106.

66. Feist M, Köstlin R, Nuss K. Claw surgery in cattle: the benefit of perioperative analgesia in recipients of embryo transfer. J Vet Med A Physiol Pathol Clin Med. 2014;61(1):4–9.

67. de Oliveira FA, Luna SPL, de Amaral JB, Rodrigues KA, Sant’Anna AC, Feist M, Köstlin R, Nuss K. Administration before laparoscopic abomasopexy to correct left displaced abomasum on markers of stress in dairy cows. J Dairy Sci. 2020;103(11):1713–22.

68. Wagner BM, Sertic DM, Schlageter P, Boll R. Non-steroidal anti-inflammatory drugs: Pharmacokinetics and mitigation of procedural pain in cattle. Animals. 2020;10(11):2245–64.

69. Coetzee H, Apley M, Pharmacokinetic-pharmacodynamic modeling of analgesic drugs in beef cattle. In: American Association of Bovine Practitioners Proceedings of the Annual Conference. 2008. pp. 29–34.

70. Coetzee JF, Nutsch AL, Barbur LA, Bradburn RM. A survey of castration methods and associated livestock management practices performed by bovine veterinarians in the United States. BMC Vet Res. 2010;6(1):1–19.

71. Johnstone ECS, Coetzee JF, Pinedo PJ, Edwards-Callaway L. Current attitudes of veterinarians and producers regarding the use of local and systemic analgesia in beef and dairy cattle in the United States. JAVMA. 2011;238(6):767–76.

72. Fajt VR, Wagner SA, Norby B. Analgesic drug administration and attitudes about analgesia in cattle among bovine practitioners in the United States. JAVMA. 2011;238(6):767–76.

73. Kasimanickam RK, Hall JB, Estill CT, Kastelic JP, Joseph C, Abdel Aziz RL, Nak D. Flunixin meglumine improves pregnancy rate in embryo recipient beef cows with an excitable temperament. Theriogenology. 2018;107:70–7.

74. Mashaghi A, Marmalidou A, Tehrani M, Grace PM, Pothisoulakis C, Dana R. Neuropeptide substance P and the immune response. Cell Mol Life Sci. 2016;73(22):4249–64.

75. Mashaghi A, Marmalidou A, Tehrani M, Grace PM, Pothisoulakis C, Dana R. Neuropeptide substance P and the immune response. Cell Mol Life Sci. 2016;73(22):4249–64.

76. Honore P, Rogers SD, Schwai MJ, Salak-Johnson JL, Lugur NM, Sabino MC, Clohisy DR, Mantyh PW. Murine models of inflammatory, neuropathic and cancer pain each generate a unique set of neurochemical changes in the spinal cord and sensory neurons. Neuroscience. 2000;98(3):585–98.

77. Ebner K, Muigg P, Singewald G, Singewald N. Substance P in stress and anxiety: NK-1 receptor antagonism interacts with key brain areas of the stress circuitry. Ann N Y Acad Sci. 2008;1144:61–73.

78. Ondrio TM, Springos DD, Singewald G, Singewald N. Substance P in stress and anxiety: NK-1 receptor antagonism interacts with key brain areas of the stress circuitry. Ann N Y Acad Sci. 2008;1144:61–73.

79. Michelgard A, Appel L, Pissiota A, Frans O, Langstrom B, Bergstrom M, Fre-Rickson M. Symptom provocation in specific phobia affects the substance P neurokinin-1 receptor system. Biol Psychiatr. 2007;61(8):1002–6.

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