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On the Cover

Schematic design of Ca homeostatic mechanisms in dairy cows in response to Ca outflow into milk during very early lactation. See page 1.
Illustration: Taraneh Ebnalnassir

Iranian journal of Veterinary Science and Technology (IJVST) is a peer-reviewed and multi-disciplinary journal that supports important research advances in veterinary medicine and subject areas relevant to veterinary medicine including anatomy, physiology, pharmacology, bacteriology, biochemistry, biotechnology, food hygiene, public health, immunology, molecular biology, parasitology, pathology, virology, etc. Contributions related to clinical sciences including large and small animal medicine, poultry disease, diseases of equine species and aquaculture are welcomed. Articles can comprise research in basic sciences, as well as applied veterinary findings and experimental studies and their impact on diagnosis, treatment and prevention of diseases.
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Subclinical Hypocalcemia in Dairy Cows: Pathophysiology, Consequences and Monitoring

Hesam A. Seifi, Samuel Kia

Keywords
milk fever, hypocalcemia, subclinical, dairy cow, monitoring

Abstract
Milk fever and subclinical hypocalcemia are the most important macro-mineral metabolic disorders that affect transition dairy cows. Many studies have shown that cows with subclinical hypocalcemia are also prone to many diseases and disorders. The drain of Calcium (Ca) during early lactation represents a significant increase in Ca demand over that for late fetal growth and physiological maintenance. The requirements of the mammary gland for Ca often exceeds the ability of the cow to replenish the plasma Ca pools. Blood Ca concentrations remarkably decline in dairy cows around calving, with the lowest concentrations occurring about 12 to 24 hours after calving. To maintain Ca homeostasis after calving, at the start of lactation, Ca compensating mechanisms are activated.

These mechanisms involve a coordinated effort among the hormones 1,25-dihydroxyvitamin D3, parathyroid hormone (PTH), and calcitonin. Hypocalcemia is associated with an increased risk of several important health conditions such as mastitis, retained placenta, metritis, abomasum displacement and immune insufficiency, particularly in transition period. The incidence of subclinical hypocalcemia approaches 40-50% in multiparous cows after calving in dairy herds. In spite of developments in preventive approaches, tremendous economical impact of hypocalcemia on health, production and fertility of dairy cows is a major concern for dairy herd owners. The paramount advances in dairy health have been the paradigm shift from treatment of clinical illness to disease prevention and redefining disease more broadly, to include subclinical conditions. Herd-based tests are now available for use in routine herd monitoring and for investigating dairy herds with metabolic subclinical problems. This review provides the criteria for hypocalcemia monitoring and interpretation of the results in dairy herds.
Introduction

During the last decade, dairy industry has undergone a wide range of major changes. Milk production has steadily risen in average production per cow and substantially in greater total annual milk production. The modern high-producing dairy cow will produce about 40 to 50 kg of milk per day in early lactation; and production as high as 60 kg/d is not uncommon [1]. This sharp and tremendous increase in milk yield has imposed great impact on metabolic capacities of dairy cows [2]. Metabolic disease incidence typically escalates as milk production rises and as herds become larger [3]. Metabolic diseases of dairy cows are manifestation of the cow’s ability to cope with the metabolic demands of high milk production. The etiology of those metabolic diseases can be traced back to insults that occur during transition period.

The biggest advance in dairy health in the last 25 years has been the paradigm shift from treatment of clinical illness to disease prevention. Health management has been defined as the promotion of health, improvement of productivity, and prevention of disease in cows within the economic framework of the owner and industry. Another major advance has been redefining disease more broadly, to include subclinical conditions (e.g., subclinical hypocalcemia, subclinical mastitis, ketosis) [4].

Milk fever and subclinical hypocalcemia (total blood Ca ≤ 2.0 mmol/L) are the most important macro-mineral metabolic disorders that affect transition dairy cows [5]. On average 5-10% of dairy cows succumb to clinical milk fever [6], with the incidence rate of subclinical hypocalcemia has been recorded at 30-50% on the day of calving [7, 8]. Both forms are associated with an increased risk of mastitis, retained placenta, endometritis, slower uterine involution, delayed first ovulation after calving, ketosis, displaced abomasum and reduced gastrointestinal motility [5]. Therefore increased incidences of these conditions suggest there may be a Ca problem in transition cows. The objective of this review article is to cast light upon the recent advances and findings in physiopathology and consequences of hypocalcemia and also will focus on strategies for testing and monitoring subclinical subclinical hypocalcemia.

Calcium homeostatic mechanisms

Ca is essential for life in animals. It is involved in many fundamental biological processes in the body, such as bone formation, muscle contraction, nerve transmission, and blood clotting, and it serves as a second messenger regulating the actions of many hormones. Therefore, it is of major importance that Ca concentration is regulated within a narrow range [9, 10]. In mammals, this process involves a coordinated effort among the hormones 1,25-dihydroxyvitamin D3, parathyroid hormone (PTH), and calcitonin [10]. Homeostatic control of Ca concentrations in blood is so strong that variations are small and do not reflect dietary intake at all [11].

The skeleton of a 600-kg cow contains approximately 8.5 kg of Ca. There are 3 g Ca in the plasma pool and only 8 to 9 g Ca in all the extracellular fluids of a 600-kg cow. Blood Ca in the adult cow is maintained between 2.1 and 2.5 mmol/L (8.5 and 10 mg/dL) and is slightly higher in young animals [12]. About 50% of the blood Ca is bound to proteins such as albumin, less than 10% is in mineral complexes with inorganic phosphates and the remainder exists in the ionized form [13]. The ionized Ca concentration is the biologically active form of the Ca in blood [14] and is most important for immediate metabolic function [15]. During acidosis, larger numbers of protons compete with Ca (and with other cations) for binding to anionic sites of plasma proteins such as albumin. This drives more protein-bound Ca into solution, thereby increasing the ionized Ca concentrations. Conversely, alkalosis decreases the ionized Ca concentrations [13]. Cows that are alkalotic from upper gastrointestinal obstruction or other conditions may have normal total Ca while exhibiting clinical signs of hypocalcemia [15]. Under acidic conditions, the ionized portion of Ca in the blood is closer to 48%; under alkaline conditions, it is closer to 42% ionized. The final 3% to 7% of Ca in blood is bound to soluble anions, such as citrate, phosphate, bicarbonate, and sulfate [12]. If total serum proteins are greatly reduced (hypalbuminemia), it is possible to have low total Ca in the blood and relatively normal levels of ionized
Ca in the blood. Appropriately measured ionized Ca is the gold standard to evaluate physiologically active forms of Ca in a patient. Adjusted formulas were suggested to correct Ca concentrations in regard to albumin or total protein variations in dairy cows, when total Ca is measured [16]. However, serum total Ca concentration has routinely been the primary source for evaluation of Ca abnormalities in dairy cows.

Dairy cows in early lactation producing colostrum (containing 1.7-2.3 g Ca per kg) or milk (containing 1.2 g Ca per kg) [9] typically secrete 30 to 40 g of Ca each day. The total amount of Ca in plasma and extracellular fluids is estimated to be 12 g. Thus, body experiences negative Ca balance with initiation of lactation in dairy cows.

Blood Ca concentration remarkably declines in dairy cows around calving, with the lowest concentrations occurring about 12 to 24 hours after calving [17, 18]. Blood samples obtained at this time can reveal the extent of hypocalcemia experienced by a dairy herd [12,19].

The drain of Ca during early lactation (30–50 g/day) represents a significant increase in Ca demand needed for late fetal growth (10-15 g/day) [20, 21]. During the dry period, the supply of Ca through the diet is usually more than requirements of dam and fetus. Thus, passive Ca transfer from intestine is adequate to maintain homeostasis without activating the Ca mobilization system, which is usually not activated until parturition [12, 19]. The main site for Ca absorption is assumed to be the small intestines, at least at moderate Ca intakes [22]. The capacity for cows to absorb Ca through the rumen and abomasum is uncertain [23, 24].

The demand of the mammary gland for Ca often exceeds the ability of the cow to replenish the plasma Ca pools, resulting in an acute decrease in the plasma Ca concentration in most cows [20]. To maintain Ca homeostasis after calving, at the start of lactation, Ca-maintaining homeostatic mechanisms are activated. These mechanisms include: increased Ca reabsorption in the kidneys, increased Ca absorption in the intestine and Ca withdraw from bone [17]. Two hormones, 1,25 dihydroxy cholecalciferol (1,25(OH) D3) and parathyroid hormone (PTH) are involved in each of these processes (Figure 1).

**Parathyroid hormone (PTH)** - As Ca concentrations in the plasma decline below 10 mg/dL, the parathyroid glands are stimulated to secrete PTH [10]. PTH is an 84 amino acid peptide that binds to receptors located on the surface of its target tissues. The primary target cells are bone osteoblasts and osteocytes as well as renal tubular epithelial cells [12].

Bone Ca mobilization and renal tubular ab-

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*Figure 1*

Ca homeostatic mechanisms in dairy cows in response to Ca outflow into milk during very early lactation (illustration by Taraneh Ebnalnassir).
sorption is enhanced by PTH. Release of PTH quickly promotes renal tubular reabsorption of Ca from the glomerular filtrate. But only small amounts of Ca are lost in urine (<1 to 2 g/day in the cow), so this action of PTH is sufficient to restore normal blood Ca concentration only if the Ca deficit is small [17]. PTH receptors are found on the osteoblasts that line all bone surfaces. Larger Ca deficits cause prolonged secretion of PTH (hours to days), which stimulates osteoclastic resorption of bone Ca to use skeletal Ca to make up the deficit [19]. The osteoblasts respond to PTH by secreting cytokine factors [25], which cause pre-existing osteoclasts to begin to resorb bone collagen to release Ca and cause osteoclast progenitor cells to differentiate and become new osteoclasts. It generally takes several days for osteoclastic bone resorption to become fully active [26].

The dairy cow is programmed to go into a state of lactational osteoporosis, mobilizing bone Ca to help her achieve normocalcemia in early lactation [17]. This will typically result in loss of 9–13% of her skeletal Ca in the first month of lactation [27], which is reversible in later months of lactation.

**Vitamin D** - A second hormone, 1,25(OH)$_2$D3, is required to stimulate the intestine to efficiently absorb dietary Ca. Two modes of intestinal Ca absorption have been proposed: one is a passive and paracellular mode that occurs at luminal concentrations of Ca > 2 – 6 mmol/L and the other mode is an active transcellular process via the action of the active form of vitamin D [28]. This hormone is made from vitamin D by the kidneys – but only in response to an increase in blood PTH [17]. The final hydroxylation of vitamin D in the kidney is regulated by PTH [21]. PTH -elevated 1α-hydroxylase enzyme activity in kidney and this enzyme have important roles in the conversion of 25(OH)$_2$D3 to 1,25(OH)$_2$D3. In addition to PTH, low blood Ca, can activate mitochondrial 1α-hydroxylase in the kidney to convert 25(OH)$_2$D3 into 1,25(OH)$_2$D3 [19]. On the other hand, high level of blood inorganic phosphorus (IP) inhibits the action of 1α-hydroxylase and production of active form of vitamin D. During late gestation, 1,25(OH)$_2$D3 is elevated 2- to 3-fold in most species [29-31] and, during early lactation in the bovine, can reach concentrations 10-fold above normal for 2–3 days postpartum [32].

Metabolic adaptation mechanisms for Ca are not rapid enough at the onset of lactation; cows require about to 1 to 2 days to maximize Ca intake from the gastrointestinal tract and from bone to the mammary gland. Consequently, almost all cows experience some degree of hypocalcemia during the first days after calving but plasma Ca concentration returns to normal within 2 to 3 days [9, 12, 19].

**Factors disrupting Ca homeostasis**

**Age** - Age has a profound effect on susceptibility of dairy cows to hypocalcemia. Older cows are affected by hypocalcemia more common and more severe than young cows. The Ca output, however, does not explain the increase in prevalence of hypocalcemia within multiparous cows with increasing age as their colostrum yield is not different [33]. The age and parity-associated susceptibility might be related Ca homeostatic mechanisms. With increasing age, the hemostasis process is impeded and results in moderate to severe hypocalcemia. It has been assumed that the number of vitamin D receptors in intestines decline with increasing age [34]. In addition, as animals age increase, the number of receptors for PTH on target tissues decline [35].

**Metabolic alkalosis** - Current evidence suggests that milk fever and hypocalcemia may occur in cows as a result of excessive dietary cations. The typical ration of dairy cows are rich in cations (primarily potassium and sodium) and has lower amounts of anions (chloride and sulfur). These cations induce a metabolic alkalosis in the cow that impairs Ca homeostatic mechanisms via attenuated responsiveness of tissues to PTH [36-39]. It has been shown that cows in a more acidotic state, which can be measured by the pH in the urine, have a decreased risk of developing milk fever [40].

In cows fed diets rich in cations, a greater number of positively charged cations enter the blood than negatively charged anions and results in an electrical disparity. To restore electroneutrality to this positively charged blood, a positive charge in the form of a hydrogen ion (H$^+$) is lost from the blood compartment and the pH of the blood is increased. Adding readily absorbable anions to the diet increases the total negative charges in the blood, allowing more H$^+$ to exist and a decreased blood pH, restoring tissue sensitivity to PTH [9].

Under normal conditions, PTH released in response to hypocalcemia interacts with its receptor, located on the surface of bone and kidney cells. This stimulates G-proteins and adenylate cyclase
resulting in production of cyclic AMP, which acts as a second messenger within the cytosol of target cells. This initiates mechanisms such as bone Ca resorption and renal production of 1,25(OH)\textsubscript{2}D\textsubscript{3} to restore blood Ca concentration to normal levels. In alkalotic conditions induced by high cationic diets, the shape of the PTH receptor protein is changed so that it is less able to recognize and bind PTH, resulting in failure to activate the cell by producing cyclic AMP. Furthermore, Mg is required for full function of the adenylate cyclase complex [17].

**Hypomagnesemia** - Hypomagnesemia affects Ca metabolism by reducing PTH secretion in response to hypocalcemia, and by reducing ability of PTH stimulated cells to produce cyclic AMP, resulting in failure to activate the target tissues to PTH [9, 17]. On the contrary, serum Ca and magnesium concentrations are negatively associated. Cows suffering from hypocalcemia have higher serum magnesium concentration [33]. In a period of low serum Ca concentration, PTH is secreted into the blood. PTH secretion raises the threshold for renal magnesium excretion, resulting in a higher serum magnesium concentration [17, 41].

**High dietary Ca** – Before calving, the approximate daily requirement for Ca is only 30 g, comprising 15 g in fecal and urinary loss and 15 g to fetal growth [21]. When supplying Ca far in excess of the daily requirements at dry period, the passive transfer of Ca is sufficient to overcome the needs of the cow and the fetus. Therefore, active hemostatic mechanisms of absorption and resorption of Ca become depressed. As a consequence, at calving when sudden massive demands for Ca occur, the cow is unable to rapidly return to hemostatic mechanisms and is susceptible to severe hypocalcemia until these mechanisms can be activated, which may take several days [42].

**High dietary phosphorus** – In dry cows, high dietary levels of phosphorus (more than 0.5% dry matter intake) increase the serum level of inorganic phosphorus, which has inhibitory effect on the renal enzyme (1α-hydroxylase) that catalyzes the conversion of vitamin D into its active form (1,25(OH)\textsubscript{2}D\textsubscript{3}) and thereby predisposes cows to hypocalcemia [43-45]. High dietary phosphorus has also been reported to have a negative affect on intestinal magnesium absorption, which further makes periparturient cows susceptible to hypocalcemia [9, 46].

**Definition and incidence of hypocalcemia**

Hypocalcemia may be clinical or subclinical. Clinical hypocalcemia, also known as milk fever, is a particular concern in the newly calved cow. Most of cases of milk fever occur within 24-48 hours of calving. Generally, cows with milk fever are recumbent and are unable to rise as a result of low blood Ca, whereas cows with subclinical hypocalcemia have no clinical signs [47].

The diagnosis of subclinical hypocalcemia is usually based on serum levels of Ca, and cut-off points at total Ca levels of 2.0 mmol/L (8 mg/dL) [8] and 1.88 mmol/L (7.5 mg/dL) [26] have been suggested. Such levels commonly occur in dairy cows soon after calving [8].

The overall incidence of milk fever found from a national dairy study in United States was 5% [8, 12]. Other field studies reporting incidence of milk fever from 1977 to 2007 found that the incidence in 10 North American studies was 3.45% (range 0–7%), in 10 European studies it was 6.17% (range 0–10%), and for 10 Australasian studies it was 3.5% (range 0–7%) [21]. Clinical milk fever was prevalent in 1, 4, 6, and 10% of first, second, third, and ≥ fourth lactation cows, respectively [48]. Prevalence of clinical milk fever in a recent German study was higher (13.4, 15.0, and 21.7% for fourth, fifth, and sixth parity, respectively) [33]. It is assumed that preventive strategies are more common in the United States. German farmers favored oral Ca supplementation more than anionic salts as a preventive strategy. They supplemented Ca subcutaneously or orally in 46.1% of the herds and only 8.7% of the herds used anionic salts to prevent hypocalcemia [33]. Based on the US agriculture ministry report, 68.9 and 27.6% of the herds used Ca products and anionic salts, respectively. The same report indicated that 20.7% of heifers and 27.6% of cows were fed anionic salts [49].

The incidence of subclinical hypocalcemia – blood Ca values between 2 and 1.38 mmol/L (8 and 5.5 mg/dL) during the periparturient period – is around 50% in older cows [50]. In a recent German study, 47.6% of multiparous cows suffered from subclinical hypocalcemia (less than 2 mmol/L) within 48 h after parturition [33]. This finding is in agreement with previous studies [8, 51, 52]. Subclinical hypocalcemia increased with age and was present in 41%, 49%, 51%, 54%, and 42% of 2nd–6th lactation cows, respectively (Figure 2) [8].
Forty percent of multiparous cows were subclinical hypocalcemic (blood Ca concentration of 2.0 mmol/L) in a five-year study on commercial dairy farms in Iran, which had annual milk production ranged from 10600 to 13000 kg (Figure 3). All the cows received anionic-supplemented rations in close-up period and sampled between 12 to 48 hours after calving (Seifi, HA; unpublished data).

In contrary to multiparous cows, there are conflicting reports on the prevalence of hypocalcemia in primiparous cows. Reinhardt et al. (2011) observed a prevalence of 25% from 480 herds in the United States [8]. However, hypocalcemia was infrequently found in primiparous cows (5.7%) in a German study [33], which is in agreement with a previously described prevalence of 2% for primiparous cows from 7 herds in Canada using the same threshold [52].

**Pathophysiology of hypocalcemia**

Ca is necessary for proper function of a wide variety of systems in the body from structural functions such as bone and other tissues to intracellular processes as a second messenger. Extracellular Ca is necessary for muscle contraction, nerve impulses, blood clotting, and is a component of milk and bone. Intracellular Ca is involved in second messenger systems for a wide variety of processes [53]. Hypocalcemia is considered as a gateway disease (Figure 4) and predisposes the cow to various metabolic and infectious disorders in early lactation [17] such as metritis [54], mastitis [55], abomasal displacement [56], and reproduction disturbances [57].

**Immune suppression** - It is well documented that nearly all dairy cows experience some degree of immune suppression during the transition period [58-61]. Contributing factors for immune suppression in transitional period consist of decreased polymorphonuclear leukocytes, glycogen stores, decreased blood Ca concentration and increased non-estrified fatty acids (NEFA) and β-hydroxybutyrate (BHBA) [58].

Ca is critical for proper immune cell function, which is very important in transition dairy cows. In a study by Martinez et al. (2012), numbers of neutrophils were reduced and their ability to undergo phagocytosis and oxidative burst was impaired in cows affected by hypocalcemia, which might in part explain the increased risk for infectious diseases [54]. Hypocalcemia is associated with decreased intracellular Ca stores in peripheral mononuclear cells [18]. This is the cause of a blunted intracellular Ca release response to an immune cell activation signal [18, 62]. Kimura et al. (2006) concluded that intracellular Ca stores decreases in peripheral blood mononuclear cells before parturition and development of hypocalcemia. This decrease contributes to periparturi-
ent immune suppression [18]. Ca also regulates cell polarity, which is required for directional cell killing, and it is also involved in the migration of leukocytes toward chemokines in the area of inflammation [63].

Feed intake and Weight loss - It has been shown that cows with subclinical hypocalcemia have impaired rumen and abomasum motility and depressed feed intake [64-66]. This reduction in ruminal and abomasal motility will likely cause a reduction in feed intake [47] and increased weight loss in early lactation [67]. Therefore, hypocalcemia may well exacerbate negative energy balance in cows that are already underfed [47].

Ketosis – Hypocalcemia has been attributed to the occurrence of ketosis [55]. Rodriguez et al. (2017) showed that hypocalcemic cows demonstrate 5.5 greater odds of having ketosis than normocalcemic cows [68]. The exact mechanism is unknown, however, the hypocalcemia impact on feed intake and resulting negative energy balance may be a factor in promoting of ketosis. Cows with naturally occurring hypocalcemia at parturition and experimentally induced hypocalcemia had elevated concentrations of NEFA and BHBA as indicators of increased lipid mobilization [8, 54, 62].

Lipolysis - Ca is also important in adipocytes for regulating lipid metabolism and triglyceride storage [69]. In studies with rat and human adipocytes, increased intracellular Ca has been shown to have antilipolytic effects [53, 70]. It can be speculated that hypocalcemia may deplete adipocyte Ca stores, resulting in increased lipolysis.

Abomasal displacement - It has been shown that hypocalcemia increases the risk for displacement of abomasum [56, 71]. Abomasal atony due to hypocalcemia seems to be a logical risk factor for abomasal displacement. Hypocalcemia may reduce abomasal tone and result in gas accumulation [65, 72, 73]. It was reported that 82% of the cows with displaced abomasum had Ca values equal or less than 2.0 mmol/L in the first week after calving [74]. There is also a report from one herd that subclinical hypocalcemia at calving was a risk factor for left displacement of abomasum [72]. Seifi et al. (2011) showed that the odds of the development of displacement of abomasum were 5.1 times greater in cows with serum Ca concentrations equal or less than 2.3 mmol/L in the first week post-partum [56]. Reduced Ca concentrations have been

Figure 4
associated with a reduction in rumen and abomasal motility, which in turn is thought to increase the risk of abomasal displacement [65]. It is likely that Ca concentration is an indicator of inadequate dry matter intake, which most likely contributes to the development of displacement of abomasum [71]. However, Leblanc et al. (2005) did not find a direct relationship between Ca concentrations and left displacement of abomasum incidence and suggested that subclinical hypocalcemia may be a function of decreased feed intake, resulting in other diseases such as left displacement of abomasum and subclinical ketosis [75].

**Dystocia, uterine prolapse and retained placent**a - Cows with clinical and subclinical hypocalcemia are at increased risk of dystocia, retained placenta and metritis [5, 47, 76]. The loss of muscle tone in the uterus due to hypocalcemia increases the incidence of dystocia, uterine prolapse and retained placenta [5]. It has been reported that milk fever affected cows are up to three times more likely to develop dystocia [47]. In some cases the increased odds of dystocia were reported as six times in hypocalemic cows than that of normal ones [55, 76, 77]. Furthermore, dystocia can increase the risk of occurrence of retained placenta.

The association of retained placenta and hypocalcemia has been reported [5, 78]. Erb et al. (1985) determined that cows with hypocalcemia were two times as likely to have retained placenta [76]. Another study showed that cows with retained placenta had lower plasma concentrations of Ca at parturition and up to 7 days after parturition than cows without retained placenta [79]. In addition, it has been reported that cows suffering from uterine prolapse have a lower serum Ca concentration than normal cows [80].

It is worthy to bear in mind that retained placenta is a multi-etiological condition with many risk factors. Therefore, any association does not mean causal relationship. On the other hand, the pathogenic process leading to retained placenta is initiated before parturition [81] and the serum level of Ca, after parturition cannot be considered a risk factor for retained placenta.

**Metritis and endometritis** – Subclinical hypocalcemia has been related to metritis [55, 82]. Because under hypocalcemic conditions, immune function may be impaired and muscle contraction diminished [83], metritis is more prone to occur [54]. Martinez et al. (2011) studied 110 cows in one herd in Florida, and demonstrated that cows with Ca <2.14 mmol/L at least once between 0 and 3 days in milk had 4.5-fold increased odds of metritis [54]. In a recent study, multiparous cows with subclinical hypocalcemia had 4.85 greater odds of having metritis compared with normocalcemic multiparous cows [68]. A significantly higher incidence rate of endometritis was observed in UK cows that suffered clinical hypocalcemia in comparison to normocalcemic cows [84].

**Mastitis** – It was reported that cows with clinical milk fever were eight times more likely to develop mastitis than normal cows [55]. Hypocalcemia reduces teat sphincter contraction, thus, an open teat canal invites environmental pathogens to enter the mammary gland. On the other hand, hypocalcemic cows tend to spend more time lying down than do normocalcemic animals, which could increase teat end exposure to environmental opportunistic organisms [47, 57, 85]. In addition, hypocalcemia has deleterious effect on peripheral blood mononuclear cells function and this exacerbates periparturient immunosuppression [18].

**Reproduction performance** – The association of clinical hypocalcemia and decreased fertility was reported in several studies [47, 68, 86]. Some reports showed that there are no differences in the incidence of uterine diseases, services per conception, or days open when comparing hypocalcemic cows with normocalcemic ones [87]. However, there are plentiful evidence indicated that hypocalcemia may cause infertility.

A UK study reported an increased number of services per conception, an increased calving to first service interval and an increased calving to conception interval for clinical hypocalcemic dairy cows [86]. Martinez et al. (2012) found that pregnancy rate and interval between calving and pregnancy were reduced under hypocalcemia [54]. It has been suggested that clinical hypocalcemia results in reduced fertility in dairy cows due to its effect on uterine muscle function, slower uterine involution [47, 86] and reduced blood flow to the ovaries [88]. Cows with clinical hypocalcemia had a greater diameter of the gravid uterine horn and non-gravid uterine horn between 15 and 45 days post-partum (indicative of slower uterine involution) and a significantly reduced likelihood of having a corpus luteum (indicative of ovulation since parturition) than normal cows [84].

Furthermore, subclinical hypocalcemia affected reproductive performance such as estrous cyclicity [89-90] and pregnancy rate to first AI.
The odds of expressing estrus before 60 days in milk were lower in subclinical hypocalcemic cows than in normocalcemic ones [68]. Caixeta et al. (2017) reported that cows with normocalcemia were 1.8 times more likely to return in estrus by the end of the voluntarily waiting period than cows classified as having subclinical hypocalcemia [91].

Higher Ca concentrations from week -1 through week 3 relative to calving were associated with increased odds of pregnancy. The odds of conceiving was 1.5 times higher for cows with pre-calving Ca >2.3 mmol/L, and 1.3 times higher for cows with Ca >2.2 mmol/L in week 1, >2.3 mmol/L in week 2, and >2.4 mmol/L in week 3 relative to calving [91].

In addition, it was reported that subclinically hypocalcemic cows have fewer ovulatory sized follicles at days 15, 30 and 45 post-partum and smaller follicles at first ovulation than normal cows [92]. It should be emphasized that trying to improve fertility in dairy herds without first having an appropriate hypocalcemia prevention strategy will bring only limited improvements.

**Culling** - An increased culling risk was reported for cows with hypocalcemia [56, 93]. Cows with serum Ca concentrations less than or equal to 1.8 mmol/L and not diagnosed with milk fever were approximately 3 times more likely to be culled in the first 60 days of lactation [59]. Seifi et al. (2011) also showed that Ca concentrations at weeks 1 and 2 post-calving were associated with subsequent culling during the early lactation period. The odds of culling in early lactation were 2.4 and 5.3 times greater in cows with serum Ca concentrations ≤ 2.2 and ≤ 2.3 mmol/L in the first and second weeks after calving, respectively [56]. Moreover, it was shown that culling risk were 4.57% and 27.6% for cows fed anionic and control diets, respectively [94].

**Milk production** - An association between low pre- and post-calving Ca concentrations and milk loss was found. The optimal Ca cut-off level was 2.1 mmol/L at week -1 and 1 relative to calving. It is likely that lower Ca concentrations are an indicator of inadequate dry milk intake rather than metabolic disease [91]. Hutjens (2003) has reported that the average loss due to milk fever per animal was the loss of 500 kg of milk [95].

The relationship between hypocalcemia and milk yield was inconsistent. Rajala-Schultz et al. (1999) found when the cows' own mid-lactation milk yield was used as a reference level, milk fever was associated with milk losses during the first 4-6 weeks of lactation [96]. On the other hand, several studies have indicated that there is no significant difference in milk yield, milk components and somatic cell count between hypocalcemic and normocalcemic cows [97-98].

In addition, it has been shown that milk protein content was lower in hypocalcemic cows at 21 and 35 DIM, but there was no difference in somatic cell count, percent milk fat, solids-non-fat, and milk yield [87].

**Herd based monitoring of hypocalcemia**

Herd-based tests are now available for use in routine herd monitoring and for investigating dairy herds with metabolic subclinical problems. It has been suggested that 12 multiparous cows to be sampled within 48 hour after calving. And, the results to be interpreted as the proportion of cows below the cut points of Ca [3, 33].

Besides defining the appropriate cut points for tests evaluated as proportion outcomes, it is also necessary to determine the alarm level for the proportion of animals below the described cut point. Because of normal biologic variation, a few individual cows are expected to be below the biologic threshold in any dairy [3].

**Ca cut-off levels** - Cows with a serum Ca concentration less than 2.0 mmol/L were considered as hypocalcemic [8, 21, 99]. Either pre- or post-partum cows with serum total Ca below 2.0 mmol/L were four times more likely to have post-partum disease problems [100]. Although this is a conservative threshold, it is well accepted in research and clinical practice [8, 21, 99].

Recently higher thresholds were associated with a negative health outcome such as displacement of abomasum and metritis [54, 91] or an increased culling risk [56, 93]. These associations, however, found in a longer risk period before or after calving [54, 56]. Neves et al. (2017) has shown that prepartum cows with Ca concentrations <2.4 mmol/L at approximately 1 week before calving to have an increased risk of being classified as subclinical hypocalcemia at parturition [101]. Martinez et al. (2012) showed that cows with subclinical hypocalcemia, as defined by serum Ca less than 2.15 mmol/L within 72 hour after parturition, had reduced concentrations of neutrophils in the blood, impaired neutrophil function, and increased incidences of metritis and puerperal
metritis compared with normocalcemic cows [54].

Furthermore, Van Saun (2000) considered serum Ca concentration below 2.25 mmol/L as a cut-off level for interpretation of hypocalcemia in a group of fresh cows whereas it would be considered normal in an individual [102]. Further evaluations are necessary to define the most appropriate threshold of hypocalcemia within any given time during transition period [33].

Interpretation of the herd based Ca testing

Because the duration of parturient hypocalcemia is extremely short (about the first 48 hours after calving), its incidence is monitored instead of its prevalence [103]. Limited data are available to assist in determining an alarm level for parturient hypocalcemia [3]. In regard to incidence of hypocalcemia, it was suggested that 30% hypocalcemia is a reasonable alarm level in multiparous Holstein cows [3].

In authors’ experience, the best time to collect blood samples is about 12 to 24 hours. Some researchers suggested cows to be sampled within 48 hours after calving [33]. Further studies are needed to determine the best sampling time and the accurate and precise cut-off level for either sampling time.

Herd's were categorized based on the proportion of positive samples (i.e., blood Ca below threshold) into negative (0 to 2 out of 12 cows), borderline (3 to 5 out of 12 cows), or positive (= 6 cows out of 12) [3, 33]. Such classification is based on the assumptions provided by Oetzel (2004) using a 75% confidence interval and an alarm level of 30% (Figure 5). The cows sampled within 48 hour after parturition and serum Ca below 2.0 mmol/L were considered as hypocalcemic [33]. It is needed to emphasize that this alarm level is considered to predict clinical hypocalcemia. Therefore, for predicting subclinical hypocalcemia, the alarm level may be lower. Some herds may be classified as borderline. In such cases, Venjakob et al. (2017) advise to draw more samples to classify the herd more appropriately [33].

Conclusion

Clinical and subclinical hypocalcemia are of great economic impact on dairy industry. Recent studies indicated that the incidence of inapparent hypocalcemia is 8-10 times greater than clinical hypocalcemia [8, 33, 48, 52]. The high prevalence of subclinical hypocalcemia should be viewed as a potential health risk to the transition cow. Culling, health problems, complications of parturition, loss of milk production and low fertility are common outcomes of hypocalcemia in dairy cows. Therefore, the economic loss due to subclinical hypocalcemia is estimated to be tremendous.

Postpartum blood Ca is below the normal range in many more cows than we have previously appreciated [8], and this is in spite of recent developments in hypocalcemia prevention. It should be highlighted that trying to improve dairy herds management without an effective prevention strat-
Milk fever and subclinical hypocalcemia can, to a large extent, be prevented by good dry cow management and appropriate nutrition. Appropriate prevention strategy includes precise monitoring of urine pH and adjusting DCAD of diets of close-up cows on the basis of urine pH results. It should be noted that the incidence of hypocalcemia is lower in primiparous than multiparous cows around calving. However, feeding anionic diets to primiparous cows seems to be beneficial, as well.

Acknowledgement

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Author Contributions

H.A.S. conceived and designed the paper. S.K. performed bibliography. H.A.S. and S.K. wrote primary drafts and H.A.S. wrote the final version of article.

Conflict of Interest

The authors declare that there is no conflict of interest.

References


68. Rodríguez EM, Arís A, Bach A. Associations between subclinical hypocalcemia and postpartum diseases in dairy cows. Journal of Dairy Science. 2017 Sep 1;100(9):7427-34.


86. Borsberry S, Dobson H. Periparturient diseases and their effect on reproductive performance in five dairy herds.
The Veterinary Record. 1989 Mar;124(9):217-9.


Histopathological changes in experimental infestation of *Paederus fuscipes* in rats

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**Keywords**

Beetle, *Paederus fuscipes*, Dermatitis, Histopathologic, Rat

**Abstract**

The genus *Paederus* consists of approximately 621 species associated with outbreaks of dermatitis. Our aim was to determine gross and microscopic changes induced by *Paederus fuscipes*. Adult *P. fuscipes* beetles were collected from infested house and then sent to laboratory. In the first group beetles were placed on the shaved parts over the shoulders of each rat. In the second group smashed insect materials were rubbed over the ear of examined animals. Gross changes after 12 hr were noticed as erythematous papules and in 72 hr the red elevated area became bigger and swollen. Microscopic examination revealed edema and mild infiltration of inflammatory cells (lymphocytes and eosinophils) after 12 hr, cell swelling and vacuolar degeneration in basal and squamous cells after 24 hr, but by 72 hr the epidermal cells were necrotic with intense accumulation of fluid and vesicles formation. Gross and microscopic changes were compared between rats exposed with squeezed beetle materials and rats exposed to live beetles. The typical gross changes were maculopapuls on the skin that histologically showed dermal edema and infiltration of lymphocytes and eosinophils.

**Abbreviations**

*P. fuscipes*: *Paederus fuscipes*

H&E: Hematoxylin and Eosin
Introduction

The *Paederus fuscipes* Curtis or rove beetle belongs to family *Staphilinidae*, order *Coleoptera* [1]. The genus *Paederus* is widely distributed worldwide and consists of approximately 621 species [2]. These beetles are usually 7-10 mm long and 0.5 mm wide when they are adult [3]. Their bright color is a warning signal for the potential predators [4]. *Paederus* beetles breed in moist areas, wetlands and salt marshes among rotting vegetation [5]. Although these insects are able to fly, they prefer to run; when running, they frequently raise the tip of their abdomen. This makes them easier to be recognized [6]. *Paederus* beetles have been associated with outbreaks of dermatitis in various countries, in Japan [7], Malaysia [8], Sri Lanka [9] and Iran [10,11]. The outbreak of *Paederus* dermatitis described both in southern [10] and Northern [11] of Iran. The *Paederus* dermatitis usually occurs in hot weather and causes numerous cases of cutaneous lesions every year. The lesions are a kind of contact dermatitis which are characterized by one or more lines of red, swollen, and blistered. The biology underlying these skin reactions are not fully understood, but inflammatory reaction is thought to be caused by a substance called pederin, when the insect is crushed on the skin. These lesions are peculiar to *Paederus* and knowing its pathological and clinical features will prevent misdiagnosis [12]. The present study was performed to determine gross and microscopic changes induced by experimental dermatitis by *P. fuscipes*.

Results

The gross appearance of the skin of rat exposed to *P. fuscipes* after 12 h showed erythematous papule and in 72 hr the red elevated area became bigger and swollen. Microscopical examination revealed edema in dermis with stratum corneum detachment from epidermis in 12 hr (Figures 1 and 2). Variable degree of epidermal spongiosis and vesicle formation with mild infiltration of inflammatory cells was evident after 24 hr (Figure 3). Squamous cell swelling eventually led to necrosis and bigger volume of fluid filled the space under stratum corneum in 72 hr (Figure 4).

Discussion

*Paederus* dermatitis may affect people of any sex, age, race, or socioeconomic status. The risk depends on the person activities and the insect habitat; exposed skin areas are at higher risk [14]. The incidence of *Paederus* dermatitis increases during the rainy season and since this organism is nocturnal, it explains the timing of skin lesions [12].

Insects produce tissue reactions by different means, some bite and some of them with spe-
Histopathological changes induced by experimental dermatitis in rats exposed to Paederus fuscipes beetles.

In this study, gross and microscopic changes of Paederus dermatitis mainly were observed in rats that exposed with squeezed beetle materials in comparison with the group that live beetles were used. The typical gross changes that were observed were maculopapuls on the skin that histologically showed dermal edema and perivascular infiltration of lymphocytes and eosinophils. The severity of lesions depends on the species. Different types of Paederus sp. are more toxic and thus produce more severe lesions. The mechanisms that lead to these skin reactions are not fully understood, but are probably caused by the release of epidermal proteases and can be concluded that type I and type III hypersensitivity reactions are involved. The pathological findings are similar to other insect bite skin reactions such as mosquito bite, black flies, louse, bed bugs, and fleas [15-18].

Similar gross signs and histological changes reported by others. Intracellular accumulation of fluid results in cytoplasmic swelling of keratinocytes, and if swelling is severe, the keratinocytes may rupture resulting in microvesicle formation [10, 19, 20]. In conclusion, this study confirmed that P. fuscipes dermatitis is induced by crushing body materials of the insect on host body.

Materials and Methods

4.1. Beetles

Wild-caught adult P. fuscipes beetles were collected from infested house from Sari, Mazandaran province of Iran. Beetles transferred into dry plastic vials containing a few fresh
grass leaves, and were covered by a lid containing several minute holes. Vials were properly kept under optimal condition at room temperature for a few days in order to maintain beetles alive, and then they were sent to the laboratory and kept in laboratory condition until use [21].

4.2. Animals

Adult male rats (250 g weight) obtained from The University of Urmia central animal breeding house were used in this experiment. Rats were anaesthetized by intraperitoneal injection of Ketamine hydrochloride (200 mg/kg). Beetle were applied to a clipped area over the shoulders of each rat and contained within gauze-covered light plastic rings (2.5 cm diameter) glued to skin [22] (Figure 5). In the second group smashed insect materials were rubbed over the ear of examined animals.

Gross changes in infested animals were recorded. Rats were euthanized 12, 24 and 72 hr after infestation and the skin samples from the infested rats were collected in 10% buffered formalin and processed for histopathology. Paraffin blocks were made; 4-5 micron sections were cut and stained with hematoxylin and eosin. They were examined under light microscope and observations were recorded.

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Author Contributions

All authors contributed to the design of study, data analysis and manuscript preparation.

Conflict of Interest Statements

The authors declare that there is no conflict of interest.

References


A survey on feline leukemia virus infection in cats in Ahvaz district, Iran: Seroprevalence and risk factors

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Keywords

Serology, prevalence, feline leukemia virus (FeLV), cat, Ahvaz.

Abstract

The purpose of the present survey was to determine the seroprevalence rate of FeLV in cats in Ahvaz district, South-West of Iran, as well as, risk factors such as age, gender, breed, lifestyle and clinical findings were evaluated. Blood samples were collected from 60 companion and 124 stray cats and antibody titers were measured against FeLV with ELISA kits. The seroprevalence was obtained 79.89% (95% CI: 74.1-85.68 percent). Chi-square test showed a significant relationship between age groups and infection ($p < 0.01$). Infection rate in cats with age below 2 years was significantly less than cats between 3-4 years ($p < 0.05$) and above 4 years old ($p < 0.01$). Also the seroprevalence was significantly higher in domestic short hair breed than Persian ($p < 0.01$). The seroprevalence was higher in stray cats than companion, nevertheless, the difference was not significant ($p > 0.05$). In conclusion, the seroprevalence was very high in cat’s population of Ahvaz district and there was a significant difference between clinical findings and serological results.

Abbreviations

DSH: Domestic Short Hair
ELISA: Enzyme-linked immunosorbent assay
FeLV: Feline Leukemia Virus
Introduction

Feline leukemia virus (FeLV) is a Gamma-retrovirus of domestic cats, a member of the oncornavirus subfamily of retroviruses. This virus is an exogenous agent with protein-core and single-stranded covered RNA that can proliferate within many tissues including bone marrow, salivary glands and respiratory epithelium [8]. The three most important FeLV subgroups are FeLV-A, FeLV-B and FeLV-C, all immunologically closely related. Only FeLV-A is contagious and passes horizontally from cat to cat in nature [8,14]. Feline leukaemia is a chronic disease which is characterized by tumoural development in hematopoietic organs as a result of oncogenic, immunosuppressive and immune proliferative effects of viral infection [8, 16]. Feline leukemia virus has a worldwide distribution and it is very common in feline population. It is a contagious virus that spreads with direct contact and can transfer via saliva and blood [26]. Virus can enter to the tissues, fluids and excretions, but its spread via urine and feces is rare. Fleas are the potential source of transfer [8]. Infection has four stages including abortive, regressive, progressive and atypical infections [25]. Cats infected with FeLV may exhibit one or more of the following symptoms. Fever, stomatitis, gingivitis, lymphadenitis, cutaneous abscess, anemia, tumors and immunodeficiency are the most common signs [6,7,12,14].

Reported prevalence differs considerably depending on the geographical region and the cat population evaluated. Rate of infection in healthy stray cats differ from 1-8% [8]. If sick stray cats are only studied, prevalence rises up to 38%. Although, prevalence is decreasing because of vaccination and application of testing and removal in most places of the world [8], nevertheless, prevalence is increasing in some areas [1]. The prevalence is related to some factors such as age, gender, health condition, life style, and geographic location [8]. The seroprevalence of FeLV was reported to be 4.8% [11], 14.2% [1], 2.2% [24] and 12.22% [28] in cats from different areas of Iran.

Several laboratory methods have been developed to detect antigen or antibody in the serum of infected cats such as PCR, ELISA, LAT (Latex agglutination test), IHA (Indirect hemagglutination assay), VN (Virus neutralization) and FAT (Fluorescent antibody test). ELISA is one of the diagnostic methods that as a screening test is effective in the detection of antigen or antibody in blood or serum samples [10,15,16]. Specificity and sensitivity of these kits (Acrolab Ltd.) are more than 95%, according to the manufacturer instructions. The purpose of the present survey was to determine the seroprevalence rate of FeLV in companion and stray cats in Ahvaz district (South-West of Iran), as well as, risk factors such as age, gender, breed, life style (companion or stray) and the possible relationship between clinical findings and ELISA results were evaluated in different groups.

Results

Our results showed that 147 out of 184 samples (79.89% and 95% CI: 74.1-85.68 percent) were positive for the presence of antibody against Feline leukemia virus by ELISA method. Statistical analysis showed a significant relationship between different age groups and infection ($p < 0.01$). Infection rate

<table>
<thead>
<tr>
<th>Category</th>
<th>Groups</th>
<th>Prevalence</th>
<th>Odds Ratio</th>
<th>95% CI for OR</th>
<th>P-Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gender</td>
<td>Female</td>
<td>76.47% (65/85)</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>male</td>
<td>82.83% (82/99)</td>
<td>1.48</td>
<td>0.72-3.06</td>
<td>0.67</td>
</tr>
<tr>
<td>Breed</td>
<td>Persian</td>
<td>37.5% (3/8)</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>DSH</td>
<td>81.82% (144/176)</td>
<td>7.5</td>
<td>1.7-33</td>
<td>0.008</td>
</tr>
<tr>
<td>Age</td>
<td>0-2 years</td>
<td>62.8% (27/43)</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>3-4 years</td>
<td>81.9% (86/105)</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>&gt;4 years</td>
<td>94.44% (34/36)</td>
<td>1.95</td>
<td>1.31-2.89</td>
<td>0.001</td>
</tr>
<tr>
<td>Life style</td>
<td>Companion</td>
<td>73.33% (44/60)</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>Stray</td>
<td>83.06% (103/124)</td>
<td>1.74</td>
<td>0.85-3.74</td>
<td>0.13</td>
</tr>
</tbody>
</table>
The present study revealed that 79.89% of companion and stray cats were seropositive for FeLV by ELISA technique in Ahvaz district, Southwest of Iran. ELISA is typically used to detect FeLV antigen or antibody within blood or serum. Consequently, use of highly sensitive methods may help to clarify the relatively high prevalence of FeLV infection. Antibody detection against FeLV is very important in cat population, because this virus is highly contagious and there are many stray cats in this area. The obtained results indicated that FeLV may be as a cause of mortality in cat’s population of this region, because of the very high prevalence. These animals can be concerned in disease transmission to other cats, particularly companion cats. Originally, certain diseases, such as lymphoma, are associated with very high rates (up to 75%) of FeLV infection [8]. Cats of these regions may suffer from such diseases. The prevalence of infection is more necessary, due to the increasing tendency for individuals to keep pets such as cats in the house and bringing feral cats inside the house. Since the vaccine is not commercially available for FeLV in Iran, the only way for prevention of the disease is the principle of hygiene and avoidance of the contacts between cats, especially stray cats [1].

Recent studies have been shown that the seroprevalence of FeLV is different in parts of the world. Rate of infection has been reported 0-2% in Sidney [18], 18% in Italy [3], 0% in South Vietnam [22] and 1% in Finland [27]. In one study in USA, the presence of FeLV antigen in pet cats was 13% [23]. The prevalence has been declared 15.6-35% in Europe [20], 2.3-3.3% in North America [17], 0-2.9% in Asia [24], 6.5-7.5% in Australia [18], 2.9-9.8% in Japan [19], 3-4.5% in turkey [30] and 3.5-10.4% in U.K [20].

In the present study, the prevalence of the FeLV infection was found to be higher than other areas of Iran. In a survey in Tehran, on 103 stray cats and healthy domestic, 4.8% were positive for FeLV and healthy domestic, 4.8% were positive for FeLV by ELISA technique [11]. In another survey by Akhtardanesh et al. (2010), overall infection rate was 14.2% for FeLV in Kerman district. Shahra-ni et al. (2011) detected FeLV infection in Iranian domestic cats by RT-PCR. Fifty six blood samples were tested using molecular methods and total frequency of FeLV infection was 2.2%. Infection rate was reported 12.22% and 65%, respectively by RT-PCR methods in other areas of Iran [21,28].

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### Table 2

Odds ratio in multivariate logistic regression based on age and breed in cats in Ahvaz district, Iran

<table>
<thead>
<tr>
<th>Category</th>
<th>Groups</th>
<th>Odds Ratio</th>
<th>95% CI for OR</th>
<th>P-Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td></td>
<td>1.86</td>
<td>1.25-2.77</td>
<td>0.002</td>
</tr>
<tr>
<td>Breed</td>
<td>Persian</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>DSH</td>
<td>5.43</td>
<td>1.18-25.06</td>
<td>0.03</td>
</tr>
</tbody>
</table>

### Discussion

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our study, the rate of infection was 79.89% which represents a serious danger for Province cat's population and shows the importance of testing and eradication of infected cats and more attention to the prevention methods such as vaccination, isolation of patient cats and sterilization. Higher prevalence than previous research probably goes back to used techniques in the diagnosis, so that the sensitivity and specificity of ELISA kits are more accurate than other methods such as agglutination and immunochromatography tests. In this research, diagnostic kits were designed to detect antibodies in blood samples. Furthermore the high rate of incidence may be due to different cut-off point. Another possible reason for the high percentage of prevalence is that we investigated the cats above one year as the prevalence is low in most cats less than 1 year. In the present survey, the rate of infection in cats with age below two years was significantly less than cats between 2-4 years and older. Cats older than 16 weeks are more likely to be infected, but cats of any age may acquire FeLV, particularly through prolonged contact [8,12]. In line with this research, Akhtar-danesh et al. (2010) showed that the prevalence of infection was higher in older cats. According to the results of Alves et al. (2011) rate of infection was under the influence of age and older cats are more likely to be infected [2]. Beatty et al. (2011), Maruyama et al. (2003) and Yuksek et al. (2005) obtained similar results [4, 20, 31].

Several risk factors may affect the prevalence of FeLV infections. Age, breed, gender, life style and health status that has been discussed associated with the prevalence of viral infections in cat's population. In this study, the seroprevalence of positive cases was more in male cats than females, but this difference was not significant statistically. Probably because males roam in the environment, is likely to be more engaged [14]. Torkan et al. (2014) showed that 63.63 percent of infections were in males; nevertheless, Hitt et al. (1992) assessed serum samples and showed that the prevalence was more in females than males [9]. Unlike the above results, the prevalence of infection were not significantly different in males (9.4%) and females (8.3%) [13]. The prevalence of FeLV is higher in cats that are allowed to roam outside, because direct contact is necessary for transmission. In a study in the United States, the prevalence was clearly related to the time spent outdoors and the degree of exposure to other cats. Of cats in a study in Boston and Detroit, which many were allowed to roam outside, 63% and 47% had positive serum FeLV antibody test results, respectively, whereas only 5% of New York cats that were primarily confined to high-rise apartments had FeLV-specific antibodies. Although fighting, free-roaming, intact male cats are still considered mainly at risk for acquiring FIV infection, the same risk factors also facilitate FeLV infection [6,8].

In the present study, the ratio of positive cases was more in stray cats than companions. In a survey conducted by Goldcamp et al. (2008), the prevalence was 7.6% and 9% among domestic and stray cats respectively. Shahrani et al. (2011) showed that domestic cats which did not have contact with stray cats were exposed to the least risk of infection. Taking care of domestic cats (such as vaccination, sterilization and blood tests for the presence of viruses) is necessary. Cats that do not have access to the outside; have less contact with seropositive cats. Neutered cats are more likely to be kept at home and are less at risk of exposure to the retroviruses. These items can be effective on the prevalence of infection [8]. The role of the cat flea (Ctenocephalides felis) has been confirmed as a vector in transmission also. The virus does not survive well in urine, feces or in the environment, so cats will not be infected just because another cat with FeLV has lived in a house before them or comes into their garden or yard. Indoor cats, which do not have contact with stray cats at all, are at minimal risk of infection [5].

It is determined that there is a significant difference between clinical findings and ELISA results. Originally, certain diseases, such as lymphoma, are associated with very high rates of FeLV infection. Of course, clinical symptoms are not specific and a high prevalence of animals that seem to have FeLV infection, are negative [8]. Our study concludes that a significant percentage of cats infected with FeLV did not show any clinical signs. As the clinical signs, are not definitive for accurate diagnosis of FeLV and on the other hand the sick cats may have not symptoms, serologic testing is recommended for all suspected cats. Clinical symptoms can include weight loss, anemia, chronic diarrhea, lymphadenopathy, oral ulcers and mucosal congestion [29,30].

In conclusion, it can be stated that ELISA technique is a sensitive and specific method for the detection of exogenous FeLV. FeLV appears to be endemic in Iran. This study highlights the need
for fast, correct and cost effective diagnostic techniques for screening healthy and sick household cats referred to Veterinary Hospitals. Our results showed that FeLV is a specific infection and the different common feline infectious pathogens seem to be endemic in cats of Ahvaz district. Testing for contamination and then prevention of contact are the most effective preventative ways between sick and healthy cats [15]. Vaccination and testing programs have proven to be effective in reducing FeLV infection and may potentially eliminate it at least in some areas. The cats should be prohibited from going outside and social behaviors like sharing food dishes and using common grounds. Regressive and progressive infections can be distinguished by repeated testing for viral antigen in peripheral blood. Bite wound is more common in intact cats than sterile cats. This study highlights the necessity of using rapid, accurate and effective diagnostic methods for screening healthy and sick household cats.

Materials and Methods

Blood samples were randomly collected from 184 cats (60 companion and 124 stray), in Ahvaz district, the capital of Khuzestan province in South-West of Iran from April 2015 to September 2016. Ketamine (10 mg/kg) and Acepromazine (0.15 mg/kg) were injected for sedative effects. The samples were collected from jugular or femoral veins and allowed to clot and centrifugated for 5 min at 1800× g. Serum was removed and stored at −20°C until assayed. Investigated parameters included putative risk factors such as age, gender, breed, life style and clinical findings. All cats were grouped with regard to housing conditions (indoor or outdoor). The age of the studied cats was between 1-7 years. Age was estimated by owner's information and dental formulary. The companion cats owners were asked to fill out a questionnaire for further information about signalment. The companion cats were divided into three age groups [≤ 2 year (n=43), between 2-4 years (n=105) and ≥ 4 year (n=36)] and based on clinical findings into two groups (at least one of signs fever, stomatitis, gingivitis, cutaneous abscess, anemia, lymphadenopathy, periodontal diseases, abscess and cachexia). Eighty five of the studied cats were female and ninety nine were male. Eight cats were Persian breed and 176 were DSH (domestic short hair). Sixty cats were kept indoor and 124 outdoor. Forty seven cats had clinical symptoms but one hundred and thirty seven had no signs.

Laboratory methods

The test was carried out with a commercial indirect ELISA antibody test kit (manufactured by Acrobel Lab Ltd.). In this way, microwells of kit were coated with specific antigens. If samples had specific antibodies against the virus, they could bind to the antigens. After addition of the substrate, a colorimetric reaction appeared which could be measured by spectrophotometer at a wavelength of 450 nm. The presence of color was interpreted as the presence of antibody against the virus in sera, and the absence of color revealed the absence of specific antibody. Four microwells were allocated for negative controls in each kit. Negative (less than 0.25) and positive samples (higher than 1) were determined according to the manufacture instructions.

Statistical analysis

Statistical analyses were performed using SPSS (Version 16.0; SPSS Inc., Chicago, USA). The association between age, sex, breed, life style and clinical findings were analyzed by Chi-square test, logistic regression and McNemar test. Differences were considered statistically significant when p < 0.05.

Acknowledgments

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Conflict of interest statement

The authors declare no conflict of interest.

Author Contributions statement

Conceived and designed the experiments: M.P., B.M. Performed the experiments: B.M., F.Z., M.R.S.A.S., M.P. Analyzed the data: M.P. Contributed reagents/materials/analysis tools: M.P., M.R.S.A.S., M.B. Wrote the paper: M.P., B.M.

References


23. Nakamura K, Miyazawa T,
Seroprevalence of feline leukemia virus infection in cats


Detection of *Mycoplasma bovis* in bulk tank milk samples by nested PCR in Mashhad, Iran

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**Keywords**

*Mycoplasma bovis*, milk tank, Mashhad, nested PCR

**Abstract**

*Mycoplasma bovis* is a highly contagious major mastitis pathogen with multiple clinical presentations in dairy cows. This kind of mastitis does not respond to available antibiotics and actually there is no effective therapy for this infection, thus the best way of prevention and control is to diagnose and cull the affected cows in the herd. The objective of this study was to detect *Mycoplasma bovis* in bulk tank milk samples by nested PCR in Mashhad, Iran. One hundred and four fresh bulk tank milk samples from 52 dairy herds were collected four weeks apart. Mycoplasma bovis was not detected from any of them by either direct PCR on milk or after enrichment in modified Hayflick’s broth. Two other mycoplasma species were detected after enrichment and one other mycoplasma species without enrichment by mycoplasma spp. primer. Sequencing of the PCR products from two positive samples confirmed the presence of mycoplasma that were *Mycoplasma canadense* and *Mycoplasma yeatsii*.

**Abbreviations**

PCR: Polymer Chain Reaction
PBS: Phosphate Buffered Saline
SPP: species

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Introduction

*Mycoplasma bovis* is a highly contagious major mastitis pathogen with multiple clinical presentations in dairy cows [1]. Also it is the most pathogenic agent of outbreaks of mycoplasmal mastitis in cattle [1]. The most important route of transmission inside herds is milking instruments such as milking machines, teat cups and also milker’s hands [2-4]. Recent infections usually occur after the introduction of infected replacements without any sanitary precautions [2, 4, 5]. Mycoplasmal mastitis does not respond to available antibiotics and actually there is no effective therapy for this infection [1], thus the best way of prevention and control is to diagnose and cull the affected cows in the herd [2, 4]. Identification of farms with mycoplasma problem and the infected cases within a herd can be performed with culture of milk from clinical mastitis suspected cows or bulk tank milk samples. Nowadays molecular approaches such as polymerase chain reaction (PCR) with higher sensitivity, higher specificity and in shorter time periods are widely used in laboratories [6]. Mastitis due to Mycoplasma is reported from many regions in the world, however there is only one report of prevalence of this microorganism from Iran [7], which had been performed in a western state by applying only the culture method. Therefore the objective of this study was to determine the prevalence of *Mycoplasma bovis* in bulk tank milk samples of herds in suburb of Mashhad in northeastern Iran by nested PCR.

Results

*Mycoplasma bovis* was not detected from any bulk milk samples by either direct PCR on milk or after enrichment in modified Hayflick’s broth (Figure 1). Two other mycoplasma species were detected after enrichment and another mycoplasma species without enrichment by mycoplasma spp. primer (Table 2). Sequencing of the PCR products from two positive samples confirmed the presence...
of mycoplasma that were *Mycoplasma canadense* and *Mycoplasma yeatsii* (Figure 2).

**Discussion**

This cross-sectional survey of bulk milk tank from representative dairy herds in Mashhad, Iran, was specifically aimed at determining *Mycoplasma bovis* and the possible role of other causative organisms. The findings indicated that no evidence of *Mycoplasma bovis* was found using nested PCR. Several PCR assays have been described in the scientific literature for the detection of *Mycoplasma bovis* [8-12]. The nested *Mycoplasma bovis* PCR was selected for use in the survey as it was reported to have a detection limit of 5 cfu/ml of milk, was analytically specific, and had a better diagnostic sensitivity than culture [11]. In addition, the PCR assay using the primary primers [PpMB920-1 and -2] was reported to differentiate all true-positive and true-negative isolates in a blinded ring trial of different PCR systems and laboratories [13]. However, in this study for confirmation of results, we used another primer set for detection of mycoplasma spp. The results of this study showed that *Mycoplasma bovis* was not detected from any of 52 farms and other Mycoplasma spp. were detected in three of 104 bulk tank milk samples. This is in contrast with another study performed in Iran that reported high prevalence of mycoplasma mastitis [7]. This could be related to the improved mastitis control programs in farms during recent years or geographical differences in the two regions being studied with more than 1000 km distance between them. Investigations in other countries showed the between-herd prevalence of *Mycoplasma bovis* in bulk milk ranging from 0 to 8 percent in New Zealand [14], USA [15], Greece [16] and Belgium [17]. Detection of mycoplasma in bulk tank milk has been shown to be repeatable, but in our study one of the farms was negative in first sampling.

**Table 1**
The oligonucleotide primers used in this study

<table>
<thead>
<tr>
<th>Primers</th>
<th>Sequence (5′–3′)</th>
<th>Annealing temperature</th>
<th>Size of amplified product (bp)</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>16S rRNA_F</td>
<td>5-GCTGGCTGTGCTGCTAATACA-3′</td>
<td>56°C</td>
<td>1013 bp</td>
<td>19</td>
</tr>
<tr>
<td>16S rRNA_R</td>
<td>5-TGCACCATCTGCTACCTGTTAACCCT-3′</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PpMB920-2</td>
<td>5′-TTTTAGCTCTTTTTGAACAAA-3′</td>
<td>48°C</td>
<td>1911 bp</td>
<td>11</td>
</tr>
<tr>
<td>PpMB920-1</td>
<td>5′-GGCTCTCTTTAAAGATGTC-3′</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PpSM5-1</td>
<td>5′-CCAGCTCACCCCTATACTGAGGC-3′</td>
<td>54°C</td>
<td>442 bp</td>
<td>11</td>
</tr>
<tr>
<td>PpSM5-2</td>
<td>5′-TGACTCACCTTTAGACCAGCTATTTAC-3′</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Table 2**
Nested PCR and modified Hayflik’s medium culture results for detection of mycoplasma in 104 bulk tank milk samples

<table>
<thead>
<tr>
<th>Mycoplasma Spp.</th>
<th>Mycoplasma bovis (nested PCR)</th>
</tr>
</thead>
<tbody>
<tr>
<td>First sampling</td>
<td>Second sampling</td>
</tr>
<tr>
<td>First sampling</td>
<td>Second sampling</td>
</tr>
<tr>
<td>PCR on milk</td>
<td></td>
</tr>
<tr>
<td>(without enrichment)</td>
<td>0/52</td>
</tr>
<tr>
<td>With enrichment</td>
<td>1/52</td>
</tr>
</tbody>
</table>
turers than medium-sized [100–499] or small herds (<100) [7].

Materials and Methods

The central region of Mashhad has a total of 57 dairy herds which included 16625 dairy cows. To ensure accurate screening, two fresh bulk tank milk samples from 52 herds were collected four weeks apart. Samples were obtained 1 to 2 hours after milking. The milk in the bulk tank was mixed for 5 to 10 minutes. Approximately 15 ml of bulk milk was taken from the top of the tank using a sterile syringe and pipette. The sample was poured in a 15 ml screw cap sterile conical tube and transported to the laboratory on the ice.

A method previously described by Pinnow et al. [11] was used to prepare the bulk tank milk samples. Briefly, milk samples were vortexed to homogeneity. 1 ml from each sample was transferred to a 1.5 ml microfuge tube, and 500µl of sterile PBS was added. After a short vortex, the samples were centrifuged at 14000×g for 20 minutes at room temperature. The supernatant was removed and the pellet was resuspended in 1 ml of PBS and centrifuged with the same conditions but for 10 minutes. Then, the pellet was subjected to DNA extraction (GeneAll®, South Korea). The samples were pre-enriched in Hayflick’s broth as described by Baas et al. [18]. 16S rDNA gene of Mycoplasma genus was detected by PCR method using specific oligonucleotide primers shown in Table 1 [11, 19]. Amplification was performed in a final volume of 25 ml containing 10 µl of Taq DNA polymerase 2x master mix red containing; 2 mM MgCl₂, Tris-HCl pH = 8.5, [NH₄]SO₄, 4 mM MgCl₂, 0.2% tween 20, 0.4 mM dNTPs, 0.2 units/µl ampliqon Taq DNA polymerase inert red dye and stabilizer (Ampliqon*, Denmark), 0.5 mg/ml of each primer and 3µl template DNA. The PCR conditions consisted of a pre-denaturation step at 94°C for 5 min, followed by 30 cycles of 30 s at 94°C, 30 s at 56°C and 30 s at 72°C. A final extension step was performed at 72°C for 5 min. Amplified products were analyzed by electrophoresis on 1% agarose gel. DNA bands were visualized by staining with ethidium bromide and photographed under UV illumination.

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Author Contributions

Conceived and designed the experiments: B.K., P.M. Performed the experiments: M.D. Consulted for microbiology experiments: M.R. Wrote the paper: M.D., B.K.

Conflict of Interest

None of the authors of this paper have a financial or personal relationship with other people or organizations that could influence the content of the paper.

References


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Immune response characteristics of Capri pox virus vaccines following emergency vaccination of cattle against lumpy skin disease virus

Hamid Reza Varshovi, Reza Norian, Abbas Azadmehr, Nahideh Afzal Ahangaran

Keywords
- Goat Pox Virus, Sheep Pox Virus, Lumpy skin disease, IL-4, IFN-γ

Abstract

In this research immune response characteristics of two available heterologous vaccines including Gorgan goat pox virus (GPV) and Romanian sheep pox virus (SPV) vaccines against lumpy skin disease have been examined, by using the monitoring of humoral and cell-mediated immune responses in vaccinated calves in the field. The evaluation of humoral immune response showed that the neutralizing antibody titers in both vaccinated groups started at day 7 post-vaccination, then reached to the protective level at day 21 post-vaccination and persisted till 35 day post-vaccination. The neutralizing antibody titers in GPV-vaccinated calves (GVC) the ratio was higher than SPV-vaccinated calves (RVC), and on days 21 and 35 post-vaccination were significantly different \((p < 0.05)\). Also, in vitro evaluation of cellular immune responses showed that the lymphocyte proliferation index and IFN-γ and IL-4 production levels in both vaccinated groups began to increase at day 7 post-vaccination until reached to its peak at day 21 post-vaccination and decreased in the period thereafter. So that, in GVC this ratio was higher than that in RVC and was significant at day 21 post-vaccination \((p < 0.05)\). The findings show that the live attenuated GPV vaccine due to induction of high level of antibody titer and higher lymphocyte proliferation and IFN-γ and IL-4 production has a good immunogenic response, so it is considered a suitable vaccine to control lumpy skin disease.

Abbreviations
- CaPV: Capri pox Virus
- DPV: Day Post Vaccination
- GGPV: Gorgan-Goat Pox Virus
- GPV: Goat Pox Virus
- GVC: GGPV-vaccinated calves
- LSD: Lumpy Skin Disease
- LSDV: Lumpy Skin Disease virus
- RSPV: RM/65-Sheep Pox Virus
- RVC: RSPV-vaccinated calves
- SI: Stimulation Index
- SNT: Serum Neutralization Test
- SPV: Sheep Pox Virus
- TCID50: 50% Tissue Culture Infective Dose

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Introduction

Goat pox virus (GPV), Sheep pox virus (SPV) and Lumpy skin disease virus (LSDV), are the members of Capri poxvirus genus of the Poxviridae family [1, 2], which can cause significant economic losses in goat, sheep and cattle, separately. They are the most important Capri-poxviruses of animals, listed in group A diseases of The World Organization for Animal Health (OIE) [3, 4]. Lumpy skin disease (LSD) is an endemic disease in Africa in cattle [4-6], and recently through import of live cattle that carries the LSDV from endemic countries has been aggressively spreading in the European, India, south-west of Middle East and other countries [7]. Several Capri poxvirus (CaPV) vaccine strains are used for the prevention and control of LSD. These vaccines are live attenuated CaPV strains including Neethling strain of LSDV, Kenyan sheep and goat pox virus (KSGPV), Yugoslavian strain of sheep pox virus (YSPV), Romanian strain of sheep pox virus (SPV) and Gorgan strain of goat pox virus (GPV) [8-10]. According to many studies, it has been proven that CaPV strains share a major neutralizing site, so that animals are infected with one strain of CaPV family and survived from it, will be resistant to infection with any other strain. Therefore, the use of vaccine strains of CaPV derived from sheep and goat would be useful to protect cattle against LSD [8, 11, 12]. In Iran, two live attenuated strains of CaPV are used as vaccines for the control of LSD [13]. These are strains of GPV (Gorgan strain) and SPV (RM/65 strain) that are produced by Razi Vaccine and Serum Research Institute (RVSRI). So, after a recent outbreak of the LSD in west and northwest of Iran in 2014, an emergency vaccination program with heterologous existing vaccines including GPV and SPV was carried out in bovine population of the country. In many scientific studies have been reported, that inadequate protective immunity induced by LSD vaccines can cause the failure of vaccination and outbreaks of disease in cattle population after exposure to LSD virus [14, 15]. Therefore, evaluation of immune response characteristics of vaccines against LSDV in field trial is very important to assess the status of the existing vaccine strains and to select the best vaccine strain that effectively protects cattle population against LSDV. Consequently, the immune response of calves was evaluated following emergency administration of live attenuated GPV and SPV vaccine formulated by RVSRI.

The aim of this study was to evaluate the immunogenicity of two Iranian live attenuated CaPV vaccines, and how these heterologous vaccines stimulated the immune response against LSD virus. Accordingly, to evaluate humoral immunity, the antibody levels of vaccinated calves were monitored weekly to give an indication of the levels of protection expected. Also, to assess the cell-mediated immune response, the peripheral blood mononuclear cells (PBMCs) of vaccinated calves were re-stimulated in vitro by inactivated virus to identify lymphocyte proliferation and Th1-type (IFN-γ) and Th2-type (IL-4) cytokine response, to determine the relationship between cytokine levels and antibody response.

Results

Adverse reactions monitoring

Clinical examination of both RVC and GVC was daily performed, and no clinical signs of LSDV were detected in any of the calves in all time-point of the experiment. In GVC, mild local reactions in the form of redness and mild swelling was appeared, as in previous study has been shown [3, 16-18], but in RVC local reaction at the vaccination site was much lower than GVC. Rectal temperature value of all vaccinated calves was recorded daily and temperatures greater than 40°C were considered fever. The fever in the GVC was first observed in 24 h post-vaccination, and its duration was 48 to 72 h post-vaccination with intermittent low-grade fever, and one week after vaccination the rectal temperature remained within normal range until the end of the experiment.

Figure 1
Daily rectal temperatures of vaccinated calves with live attenuated GPV and SPV vaccine. Mean temperatures for all calves during the 35 days post vaccination period are shown. The upper limit of the normal temperature range (40°C) is indicated by dashed lines..
Also, in RVC slight increase in fever was observed in 24 to 48 post-vaccination and was remained within normal range until the end of the experiment. In General, the fever in GVC was higher than RVC, and persisted for up to 3 days. In a few calves of GVC during the onset time of fever, ocular and nasal discharge was observed, whereas in RVC no clinical signs were observed (Figure 1).

Neutralizing antibody titers following vaccination

Neutralizing antibody titre of all calves were negative before vaccination, but after the vaccination, the neutralizing antibodies were detected in the serum of all cases. In each vaccinated group, the first detectable neutralizing antibody titer was after 7 days and rose to peak at 21-35 days post vaccination, and in comparison to day 0 a significant difference was observed at days 7, 21 and 35 ($p < 0.05$) (Figure 2). Although, the mean of the neutralizing antibody titer between GVC and RVC at days 7, 14 and 35 post vaccination was relatively similar and there was no statistically significant difference ($p > 0.05$), though in GVC appeared slightly higher.

Lymphocyte proliferation response

Analysis of the recall immune response is found necessary to boost the vaccinated calves to aid detection of the virus-specific proliferation [19, 20]. Therefore, a memory response of immune cells was provable when the PBMCs of vaccinated calves were re-stimulated in vitro with inactivated virus [21, 22]. Blood samples had been taken from the calves before (0 day) and after (up to 5 weeks) vaccination, and lymphocyte proliferation assay were performed. Stimulation index (SI) was calculated and varied from week to week and calve to calve in each group. PBMCs of vaccinated groups did show
higher proliferation than control (non-vaccinated) group (data not shown). The mean SI of vaccinated groups in response to vaccine strains increased at 7 dpv and peaked at 21 dpv and decreased thereafter. A significant difference was found in both vaccinated groups between day 0 and days 7, 21 and 35 dpv (p < 0.05). Also, SI in GVC was higher than RVC in all weeks of experiment, and this difference was significant at days 7 and 35 (p < 0.05) (Figure 3).

**Cytokine production of stimulated PBMCs**

Supernatants from the stimulated PBMCs were analyzed for the presence of cytokine proteins by ELISA technique. The Th1-like cytokine, IFN-γ, and the Th2-like cytokine, IL-4, was found in variable levels in the supernatant of virus-stimulated PBMCs in both GVC and RVC, and were significantly increased when compared with the unstimulated cultures (control group) at all-time points (data not shown).

The median values of IFN-γ and IL-4 production of each vaccinated groups demonstrated a wide range of values, and increased at 7 dpv, peaked at 21 dpv and decreased in the period thereafter. In the GVC a significant difference was found between day 0 (19 ± 7.29 pg/ml) and days 7 (33.02 ± 4.42 pg/ml), 21 (65.06 ± 9.81 pg/ml) and 35 (39.50 ± 5.75 pg/ml) dpv for IFN-γ (p < 0.05), and between day 0 (50.77 ± 23.59 pg/ml) and days 21 (166.52 ± 32.58 pg/ml) dpv for IL-4 (p < 0.01) (Figure 4A).

Also, in RVC this difference only was observed between day 0 (13.23 ± 2.63 pg/ml) and days 21 (44.07 ± 10.15 pg/ml) and 35 (38.03 ± 10.22 pg/ml) dpv for IFN-γ (p < 0.05), and between day 0 (44.78 ± 5.87 pg/ml) and days 21 (93.29 ± 15.04 pg/ml) dpv for IL-4 (p < 0.01) (Figure 4B).

In general, the IFN-γ and IL-4 production in GVC was higher than RVC at all time points of the experiment, and the significant difference between the groups was only detected at 21 dpv (p < 0.05).

**Discussion**

In this research we sought to provide detail on the immune response characteristics of the GGPV and RSPV vaccines by measurement of specific antibody and target cytokines, because they are critical parameters in immune response and can be related to the durability of protection.

Rectal temperature and local reactions in the form of redness and mild swelling at the vaccination site in GVC was higher than RVC, however, there was no statistically significant difference (p > 0.05) [3, 16-18]. The recorded clinical signs were also in agreement with Diallo and Viljoen (2007), who stated that the clinical signs caused by different Capri pox viruses are very variable, depending not on individual host susceptibility, but also on the virus strain. In cases where the local reactions at the vaccination site were very low or not observed, may also indicate that the vaccine virus was over-attenuated and therefore failed to produce an effective cell-mediated immune response [10].

The result of this study showed that all calves in both vaccinated groups were able to produce antibodies in response to vaccine strains, indicating that the specific immune defenses had been efficiently induced [23, 24]. The neutralization antibody titers of vaccinated calves were increased at each day of follow up period after day 7 and increased up to day 35. These finding are consistent with results obtained in other studies, indicating that vaccinated calves produce neutralizing antibodies before day 7 after vaccination [25, 26]. Protective level (1.5) at day 21 post vaccination increased gradually to day 35. Protective level of neutralizing antibody against CaPV were considered NI ≥1.5 [4, 26, 27].

Based on previous studies, it has been proven that cell mediated immune response plays an important role against CaPV beside humoral immunity [28, 29]. Accordingly, cell-mediated immune responses were demonstrated using the lymphocyte MTT proliferation assay in which responses are probably mainly attributable to T-helper cells [30, 31], and caused by recognition of conserved epitopes within or even between serotypes having genetic relationship [21, 30]. Stimulation index of calves vaccinated-PBMCs in GVC was higher than that in RVC in all time points of the experiment, indicating high stimulatory effects of the GPV vaccine. These results were in agreement with other studies, who reported the increase of lymphocyte activity at day 3 post vaccination and reached its peak on day 10 dpv, then decreased till day 30 post vaccination [32, 33].

In many previous studies, immune responses of CaPV have been investigated, but the functional role of induced cytokines by vaccination and how they contribute to protective responses have not been clearly identified [26, 27]. Since cytokines are...
generally produced locally at low levels, they might be difficult to detect systemically; hence in vitro stimulation of cultured PBMCs with virus can be helpful to investigate virus-induced cytokine production. IFN-γ and IL-4 are the most important cytokines in the host defense against infection by viral and microbial pathogens and their activities can directly inhibit the viruses.

In this study after re-stimulation of calves vaccinated-PBMCs with vaccine strains, the production of IL-4 and IFN-γ cytokines were observed and a significant difference between the groups was detected, so that, this increase in GVC was higher than RVC and was significant at 21 dpv (p < 0.05). From the above results it can be deduced that GGPV vaccine provides good immunogenicity, inducing a higher level of IL-4 and IFN-γ than RSPV vaccine. These results were in agreement with other studies, who detected that experimentally infected calves produced serum IFN-γ, IL-4, IL-12 and other pro-inflammatory cytokines [34].

Conclusion

In this study experimental challenge post vaccination was not performed to evaluate the efficacy of the induced cellular and humoral response in protecting the calves against LSDV invasion. However, there is an argument that protective level of neutralizing antibody against LSDV challenge is NI ≥1.5. Based on this study we conclude that GGPV and RSPV heterologous vaccines against LSDV induced humoral and cell mediated immune response, with the induction of both a Th1-like and a Th2-like activity. GGPV vaccine due to inducing high level of antibody titer and IL-4 production level, and also higher lymphocyte proliferation and IFN-γ production level against LSD virus is considered a suitable vaccine to control the disease in the field.

Materials and methods

Animal experiments

We used 48 Holstein breed male calves with approximately 4-6 months of age from two dairy farms (each farm 24 calves). Random sampling method (RSM) was used to select the study calves from each farm and to vaccinate with the vaccine strains. The calves on each farm were divided into two groups; vaccinated calves (20 calves) as a treated group and unvaccinated calves (4 calves) as a vaccine control group.

Types of Vaccine and Vaccination study

Capri pox virus vaccine-induced immunity

Gorgan-goat pox virus (GGPV) and Romanian-sheep pox virus (RSPV) which produced by the Razi vaccine serum research institute (RVSRI) of Iran were used. These vaccines were live attenuated, lyophilized and one dose of each vaccine for goat and sheep contained 105.2 TCID50/ml of virus. Ten-fold dose of vaccines was prepared according to the manufacturer’s instructions for emergency use against LSD in cattle [13]. In the vaccination study, in order to confirm that the calves were free from maternal antibody against lumpy skin disease virus, serum neutralization test (SNT) were used. Treated groups in each farm were vaccinated with live attenuated GGPV- and RSPV-vaccines, respectively, and the control groups received PBS alone. These calves were received 5 ml volume of 10-fold dose of vaccine subcutaneously (SC) according to the manufacturer’s instructions. All immunized calves were daily examined for any increase of rectal temperature and appearance of adverse reactions for 35 days post vaccination.

Virus preparation

The viruses (vaccine strains) was obtained from RVSRI and was used to make a master stock of the virus, and also, to eliminate a potential source of variability, a single batch of virus was used. SPV and GPV virus cultivation was carried out according to the standard protocol of the Department of Animal Viral Vaccines of RVSRI following OIE manual [4, 13].

Briefly, the monolayer cell lines (LK) were prepared in test tube by using DMEM and Hanks media that contained 5-10% fetal calf serum (FCS). Ten-fold dilution of viruses (10⁻¹ - 10⁻⁵) was prepared in Hanks solution. Then, 0.1 ml of each dilution was inoculated into prepared cultures tubes. The tubes were incubated at 37°C and examined for the presence of CPE till 10 days post inoculation and the titer of stock prepared virus, calculated by Reed & munch method [35]. For purification and inactivation of viruses, after the removal cell debris, harvested virus was concentrated by ultracentrifugation in sucrose density gradient (36%), and after the titration, the virus inactivation was carried out according to OIE manual [22].

Antibody titration

The serum neutralization test is the gold standard to detect antibodies against CaPV [4, 36, 37]. Serum Samples were collected on days 0 (pre-vaccination), 7, 21 and 35 post-vaccination (dpv) and the neutralizing index (NI) was measured in a micro-neutralization assay, according to the standard protocol of RVSRI institute following OIE manual [4].

PBMCs Isolation

Whole blood was taken weekly from the jugular vein of calves and were collected in sterile heparin tubes. Peripheral blood mononuclear cells (PBMCs) were isolated within 4-8 h by density gradient centrifugation through Ficoll-Hypaque solution (Histopaque-1077), according to standard protocol [31, 38]. Viable and dead cells percent was determined by staining with trypan blue (Sigma-Aldrich, Germany) and adjusted to concentration of 2 × 10⁶ viable cells /ml in RPMI complete medium [39, 40].

Lymphocyte proliferation assays

To evaluate the extent of the cell-mediated immune response, vaccine strains-and LSDV-induced in vitro lymphocyte proliferation response for each vaccinated and control
Cytokine assays

Functional analysis of the effector cells generated upon activation of PBMCs with vaccine strains should contribute to the elucidation of the basic mechanism responsible for the stimulation of the immune system [21, 29]. For this purpose, cytokine production analyses focused on the secretion of Th1-like cytokine, IFN-γ, and Th2-like cytokine, IL-4, because they play an important role in humoral and cellular immune response against infectious agents. Concentrations of IL-4 and IFN-γ cytokines in supernatants of vaccine strain-stimulated PBMCs were measured for each vaccinated group. Cell-free supernatants were collected on the fourth day of culture and analyzed for cytokines concentration. My previous experiments had shown that IFN-γ and IL-4 concentrations in the supernatant were optimal at this time point [31, 39]. All samples were stored at -70°C until analysis and concentrations of IFN-γ and IL-4 were measured by using commercially available ELISA-kits (USCN Life Science Inc., China). Assays were performed according to the manufacturer's protocol and with reference standards provided by the manufacturers and the mean values used. The limits of detection (LOD) for the individual assays were as follows: IL-4, 6.2 pg/ml and IFN-γ, 12.8 pg/ml. In each assay, a control recombinant cytokine was added with reference standards provided by the manufacturers and the limits of detection were calculated using this standard curve.

Statistical analysis

In this study, data were analyzed by the analysis of variance (one-way ANOVA) using general linear model procedures and descriptive statistics was used to quantify levels of NNT antibody titres across each sampling day. A P-value of less than 0.05 was considered significant.

Acknowledgements

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34. Nfon CK, Marszal P, Zhang S, Weingartl HM. Innate Immune Response to Rift Valley Fever...


Detection and identification of avian adenovirus in broiler chickens suspected of inclusion body hepatitis in Khuzestan, Iran during 2015-2016

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Keywords
Avian adenovirus, broiler, FADV-11, IBH, Southwest Iran

Abstract
Avian adenoviruses (AAV) are known as a very diverse group of pathogens causing a variety of clinical symptoms or being totally asymptomatic in poultry flocks. The aim of this study was the molecular detection of avian adenoviruses in broiler flocks suspected of the IBH and respiratory syndrome in the southwest of Iran. For this intent, the liver and lung samples with macroscopic lesions were collected from 30 different poultry flocks (poultry of slaughterhouse and flock mortalities). Subsequently, DNA was extracted from samples and examined using PCR. The L1 (Loop1) region of the hexon gene was amplified. PCR products were sequenced to reveal the identity of the avian adenoviruses. The data resulted from the nucleotide sequencing were analyzed using programs and services provided by National Center for Biotechnology Information (NCBI). The results showed that the pools of liver samples from a 25 days old flock were positive in the PCR test. Based on the sequence data, adenoviruses belonged to the D genotype of avian adenoviruses. In phylogenetic analysis, FADV isolates were closely related to the FADV-11 isolates of Iran, China, Canada and Australia with nucleotide homology up to 99%. This is the first study on molecular detection and analyzing the nucleotide sequence of hexon gene fragment of FADV in broiler farms in Southwest Iran.

Abbreviations
AAV: Avian Adenovirus
IBH: Inclusion Body Hepatitis
L1: Loop 1
FADV: Fowl Adenovirus
bp: Base Pair
Introduction

Adenoviruses cause a wide range of infections in the chickens all around the world. Infected farms might show high mortality rates, liver lesions (IBH), hydropericardium syndrome (HPS) and secondary infections caused by suppression of immune system. Several adenovirus strains led to lymphocyte depletion in bursa, thymus and spleen which in turn suppress the immune system [1]. Adenoviruses are categorized into four genera: Mastadenovirus (in mammals), Aviadenovirus (in birds), Adenovirus (in birds, mammals and reptiles) and Siadenovirus (in birds and amphibians) [2]. The International Committee for Taxonomy of Viruses (ICTV) proposed twelve serotypes for aviadenoviruses classification [3]. Already, FAdV known strains have been alienated into five genotype species, including A to E genotypes [1]. There exists a correlation between the FADV genotypes and serotypes. Genotype A includes FAdV1, type B: FAdV5, type C: FAdV4 and 10, type D: FAdV2, 3, 9 and 11 and genotype E contains FAdV6, 7, 8a and 8b [4]. Inclusion body hepatitis (IBH) is a significant disease in 3 to 6-week-old broiler chickens. Various serotypes of fowl adenoviruses have been detected in natural occurrences of IBH, especially those in E genotype can cause serious liver damage in IBH [5].

Currently, PCR is a rapid and sensitive technique developed for identification of avian adenoviruses. For the purpose of identifying and assigning isolates to the serotypes and species, molecular methods, especially PCR, have been being generally used [5]. The hexon (NCBI accession number: MG738474) is known as one of the major structural proteins of fowl adenoviruses. This protein is determinative for type, groups and subgroup-specific indexes. The hexon gene was chosen as the target for primer preparation in molecular diagnostic test [1]. With reference to the past studies carried out in Iran, no study has reported avian adenoviral infections in the Southwest of Iran. The aim of this study was detection and identification of avian adenoviruses in broiler chickens suspected to inclusion body hepatitis (IBH) and respiratory syndrome in Southwest Iran during 2015-2016.

Results

The PCR with degenerate primers resulted in the amplification of a 590 bp product, as expected. The results showed that one 25 days old flock with 10% mortality (3.3%) was positive for AAV (Figure 1). A phylogenetic tree (Figure 2) was constructed based on the nucleotide sequences of the L1 hexon gene and the corresponding regions of the other AAV strains rescued from GenBank. Phylogenetic analysis of this isolate revealed that they could be classified into 11 serotypes of D genotype of avian adenoviruses (Table 1). Furthermore, this is closely related to the previously reported AAV isolates such as KF406339.1 (Iran), KU981146.1, ANG57898.1, KU981145.1, KU981143.1 (China) and EF685644.1 (Fowl adenovirus D Canada) with nucleotide homology which reached up to 99%.

Discussion

Inclusion body hepatitis (IBH) has been recognized as an economically important disease occurring throughout the world [6,7,8,9]. Different serotypes of FAdV are able to cause IBH disease. In the last decade, most of the isolated serotypes which are responsible for IBH, belong to D and E genotypes [10]. In this study, the infection rate of AAV was 3.3%, which indicated the limited distribution of AAV in Ahvaz region. Nateghi et al. (2014) reported that prevalence of avian adenoviruses in broilers in northeast Iran was 10% [2]. Herdt et al. (2013) in a ten-year survey, detected Fowl adenovirus infections in 38 of 310 diseased Belgian broiler flocks [1]. Based on the L1 loop of hexon gene sequence analyzing, the IBH prevalence is usually related to the serotype 6, 7 and 8; However, the viruses correlated with those outbreaks in Iran were adenovirus serotype 11 [11]. The nucleotide sequence data of the obtained pos-
Detection and identification of AAV in broiler chickens suspected of IBH in Iran

positive sample showed that this adenovirus belonged to FAdV-11, which is in the D genotype group. Hoseini et al. (2012) identified FAdV-11 serotype from mortalities of three-week-old chicken farms [5]. They concluded that this serotype is able to cause clinical diseases and mortality in chickens. In Nateghi et al. (2014) study, FAdV 8b, 2 & 11 were identified [2]. In Herdt et al. (2013) survey, FAdV isolates belonged to the serotypes FAdV 1, 2/11, 3, 5 and 8a [1]. FAdVs correlated with IBH prevalence by hexon gene loop 1 sequencing in Canada from 2000 to 2006 were genetically linked to FAdV2, 8a and 11 [12]. Again, in 2003, the Fowl Adenovirus isolates in Ontario, Canada matched with Serotype 11 in group D genotype [13]. FAdV-11 strain 1047 was the reason for IBH outbreaks in Saskatchewan, Canada [12]. Another study in 2007 identified 18 FAdV-11 amongst 55 fowl adenovirus from different flocks in South Korea with 99% homology to 1047 strain [14]. In 2010, Fowl adenovirus serotype 11 found in Hungary [15]. Alvarado et al. (2007) recognized isolated adenovirus as Stanford strain and characterized as European Serotype 9 [16]. Since most cases of IBH are the consequences of the vertical transmission, vaccines have been proved to be a highly successful solution for controlling IBH by preventing vertical transmission and inducing maternal immunity [16,17].

In conclusion, this study confirms the presence of solely serotype 11 aviadenovirus in broiler flocks in Southwest Iran. The rate of AAV detection in broiler chicken flocks around Ahvaz was inconsiderable. As a result, detection of aviadenovirus showed that AAV is not prevalent in respiratory disease and liver lesions in Ahvaz area.

Materials and Methods

Samples

The poultry of slaughterhouse having hepatic and respiratory liver and lung involvements were evaluated. The liver and lung samples were taken from 20 different flocks; Each flock was comprisi-
ing 10,000 chickens, 10 chickens with liver and lung lesions were selected from each flock for taking samples. In addition to this, liver samples (which were enlarged, pale, showing petechiae, and ecchymosis signs) (Figure 3), were taken from 10 to 35 days old broiler chickens of 10 farms located around Ahvaz. These farms were experiencing increasing mortality (10 to 30%). Five to 10 liver samples of each farm were combined as pools. Finally, 410 samples were collected and included in this study.

It should be noted that the adenovirus vaccines are not common in broiler chickens in Iran.

**DNA extraction**

The DNA extraction from the tissue samples was performed with the Cinnapure DNA extraction kit (Cinnaclon Co., Iran). 50-100 mg of each tissue sample was cut into small pieces using a scalpel and placed into a sterile 2 ml microtube. In order to complete the homogenization process, 100 µl of prelysis buffer and 20 µl of ribonuclease were added to the microtube and it was kept at 55°C for 3 hours. During incubation time, the microtube was vortexed (for 5 seconds) at 5 minutes intervals to increase the DNA yield. Stages of the DNA extraction were carried out carefully according to the manufacturer’s instruction. Finally, extracted DNA was collected in a sterile 1.5 ml microtube and stored at -80°C for the subsequent PCR process.

**Polymerase Chain Reaction (PCR)**

The 590 bp region of the hexon gene (NCBI accession number: MG738474) was amplified using a pair of specific primers. The said primers were Hex L1-F (5’-ATGGGAGCSACCTAYTTCGACAT-3’) and Hex L1-R (5’-AAATTGTCCCRARAANCCGATCTA-3’) (2). The PCR process was carried out in a 20 µl reaction volume containing 10 µl 2x Ampliqon PCR master mix (Denmark) with 1.5 mM MgCl₂, 0.5 µl of each primer (10 Pmol/µl), 6 µl of ddH₂O and 3 µl extracted viral DNA. As such, the thermocycler (Quanta Biotech, Germany) was configured in the following conditions: 94°C for 5 minutes followed by 35 cycles of 94°C for 60 seconds, 54.2 °C for 45 seconds, 72°C for 60 seconds, and a final step at 72°C for 5 minutes. Meanwhile, in all stages of PCR, negative control (ddH₂O instead of DNA) and positive control (the DNA from Australian FAdV-8b vaccine strain (Intervet Pty Ltd.)) were utilized. The PCR products (590 bp) were separated by electrophoresis using 1% agarose gel. The RT-PCR products were cut from the gel, purified by the AccuPrep PCR Purification Kit (BioNeer Co., South Korea) according to the manufacturer’s procedure, and then sequenced in the forward direction. Sequencing reactions were performed by BioNeer Co., South Korea.

**Characterization**

In order to perform genotyping, a 590bp region of the hexon gene of the extracted positive DNA sample was amplified. Then, the nucleotide sequence was aligned and compared with those of previously identified isolates from Iran and worldwide avian adenoviruses references. The nucleotide sequences of the hexon gene determined in this study were also compared to the AAV sequence data available in the National Center for Biotechnology Information database (http://ncbi.nlm.nih.gov) using nBLAST (http://blast.ncbi.nlm.nih.gov/Blast.cgi), and the phylogenetic relationship was established by http://www.phylogeny.fr/simple_phylogeny.cgi. All of the sequences were aligned with the use of CLUSTAL W. Distance-based neighbor joining trees were constructed using the approximate likelihood-ratio test (aLRT) available in http://www.phylogeny.fr/simple_phylogeny.cgi.

**Acknowledgements**

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**Author Contributions**

Molecular detection of virus: Z.B., P.T.G.; Diagnosis of affected poultry carcases: A.R.; Scientific counseling: M.M.; Sampling: S.E.

**Conflict of Interest**

Conflicts of interest: none

**References**

2. Nateghi E., Razmyar J., Bassami M.R. Molecular char-
Detection and identification of AAV in broiler chickens suspected of IBH in Iran


Modulation of growth performance, haemato-immunological parameters, gut microbiota and stress resistance upon feeding juvenile *Schizothorax zarudnyi* (Nikolskii, 1897) by fructo-oligosaccharide

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**Keywords**

*Schizothorax zarudnyi*, *Fructo-oligosaccharide*, *Growth*, *Haemato-immunological parameters*, *Gut microbiota*

**Abstract**

A 63-day experiment was carried out under controlled conditions to compare the effects of fructo-oligosaccharide (FOS) at four levels (5, 10, 20 and 30 g/kg) on growth performance, nutritional efficiency indices, haemato-immunological parameters, stress resistance, digestive enzymes and cultivable autochthonous intestinal microbiota of juvenile (68.52 ± 1.52 g) Khaju fish *Schizothorax zarudnyi*. Fish fed the diet containing 20 g/kg FOS had significantly (*p* < 0.05) higher weight. Dietary FOS supplementation (5-20 g/kg) showed significant effects on SGR compared with control treatment. Hb, Haematocrit, MCV, MCH and lymphocytes in fish fed with the diet containing 20-30 g/kg FOS were significantly higher than those in fish fed with control treatment. After 63-day feeding period and also, 5-min air exposure challenge test, the activities of IG, LYZ and ACP in serum of fish fed with the diet containing 10-30 g/kg FOS showed a significantly higher trend than other treatments. The ratio of lactobacillus count to total autochthonous intestinal microbiota in fish fed with 10-30 g/kg FOS was significantly higher than that in other treatment groups. Furthermore, dietary FOS supplementation significantly increased survival rate of juvenile Khaju fish. Polynomial regression of SGR, FCR, PPV and PER suggested that the optimum dietary FOS level could be higher than 18.2 and < 23.8 g/kg in fish reared in culture conditions. These results indicate the beneficial effects of FOS, and emphasizes the need for further research to analyze the use of prebiotics on growth performance of fish.

**Abbreviations**

FOS: Fructo-Oligosaccharide
DP: Degree of Polymerization
SGR: Specific Growth Rate
VFI: Voluntary Feed Intake
**Introduction**

Khaju fish, snow trout, *Schizothorax zarudnyi*, is an endangered endemic species in the southeast of Iran [1]. Successful propagation and high efficiency hatching techniques in freshwaters have led to domestication process of this species during the last decade [2,3]. To determine nutrient requirements, suitable ingredients and inclusion levels in the diet of aquatic species are critically regarded as key indices to acclimatize aquatic species in captive areas [4–8]. Using feed additives (nucleotides, probiotics, prebiotics and synbiotics) in the aquaculture industry is an important option to obtain sustainable production due to increase in non-specific defense mechanisms as well as improvement of fish health status [6,8–10].

Fructo-oligosaccharide (FOS or oligofructose), a fructan existing in a number of common foods (garlic, onion, artichoke and asparagus), is a hydrolyzed product of inulin [11,12]. To achieve an integrated approach in order to opt a profitable prebiotic in the aquafeeds, it is crucial to consider main parameters including prebiotic origin (fungi or plant), chemical structure, degree of polymerization (DP), initial weight, feeding period, supplement dose, basal diet formulation, intestinal flora, type of tested biological responses including growth performance, survival rate, carcass quality, enzymatic and immunological indices and knowledge of setting up a challenge test (e.g. physical, chemical or biological stressors) [6,8,13]. In spite of conflicting results [14–16], profitable effects of dietary FOS supplementation on growth performance and survival [11,17–20], gut microbiota [19,21], immune response [Buentello et al., 2010; Hoseinifar et al., 2011] and digestive enzyme ac-
Activity [18,20] have been reported in several fish species. Since culture intensification of Khaju fish, can lead to inappropriate water quality, and to increased levels of physical, chemical and biological stressors in cultured organisms, it is important to decrease outbreaks of infectious diseases, to increase fish resistance, and to keep the high health status of fish using dietary supplements. To the best of our knowledge, there is no available information on the effects of dietary FOS supplemen-

### Table 1
Composition of the experimental diets (g/kg dry matter)

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>Control</th>
<th>Dietary fructo-oligosaccharide (g/kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>393</td>
<td>393</td>
</tr>
<tr>
<td>Soybean meal</td>
<td>146</td>
<td>146</td>
</tr>
<tr>
<td>Corn gluten</td>
<td>75</td>
<td>75</td>
</tr>
<tr>
<td>Wheat flour</td>
<td>167</td>
<td>167</td>
</tr>
<tr>
<td>Fish oil</td>
<td>54</td>
<td>54</td>
</tr>
<tr>
<td>Canola oil</td>
<td>54</td>
<td>54</td>
</tr>
<tr>
<td>Choline chloride (70%)^b</td>
<td>20</td>
<td>20</td>
</tr>
<tr>
<td>Vitamin C (stay)c</td>
<td>11</td>
<td>11</td>
</tr>
<tr>
<td>Vitamin premix^f</td>
<td>20</td>
<td>20</td>
</tr>
<tr>
<td>Mineral premix^f</td>
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<td>20</td>
</tr>
<tr>
<td>Carboxymethyl cellulose</td>
<td>40</td>
<td>35</td>
</tr>
<tr>
<td>FOS e</td>
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<td>5</td>
</tr>
<tr>
<td></td>
<td></td>
<td>10</td>
</tr>
<tr>
<td></td>
<td></td>
<td>20</td>
</tr>
<tr>
<td></td>
<td></td>
<td>30</td>
</tr>
</tbody>
</table>

**Chemical composition**

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dry matter</td>
<td>904</td>
</tr>
<tr>
<td>Crude protein</td>
<td>350</td>
</tr>
<tr>
<td>Crude fat</td>
<td>120</td>
</tr>
<tr>
<td>Crude fiber</td>
<td>94</td>
</tr>
<tr>
<td>Nitrogen free extract</td>
<td>382</td>
</tr>
<tr>
<td>Ash</td>
<td>54</td>
</tr>
<tr>
<td>Gross energy (MJ/kg)</td>
<td>18.90</td>
</tr>
</tbody>
</table>

a Behparvar Aquafeed Co, Iran.  
b Kimia Roshd Co, Iran.  
c Sigma, Germany.  
d Scharloo Chemical Co, Spain.  
e Fructo-oligosaccharide (FOS, Raftilose® P95, Orafti Co., Belgium)  
f Mineral premix contains (mg kg⁻¹) Mg, 100; Zn, 60; Fe, 40; Cu, 5; Co, 0.1; I, 0.1; Antioxidant (BHT), 100.  
Vitamin premix contains (mg kg⁻¹) E, 30; K, 3; Thiamine, 2; Riboflavin, 7; Pyridoxine, 3; Pantothenic acid, 18; Niacin, 40; Folacin, 1.5; Choline, 600; Biotin, 0.7 and Cyanocobalamin, 0.02

### Table 2
The mean (± SD) of initial weight (g), final weight (g), voluntary feed intake (% BW/day) and survival rate (%) of juvenile Khaju fish fed the experimental diets after 63 days (n=3)²

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>Dietary fructo-oligosaccharide (g/kg)</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Initial weight (g)</td>
<td>69.06 ± 0.85</td>
<td>67.29 ± 1.12</td>
<td>68.05 ± 1.15</td>
</tr>
<tr>
<td>Final weight (g)</td>
<td>86.72 ± 1.12</td>
<td>96.85 ± 1.25</td>
<td>110.45 ± 1.32</td>
</tr>
<tr>
<td>VFI (% BW/day)</td>
<td>1.82 ± 0.12</td>
<td>2.12 ± 0.08</td>
<td>2.58 ± 0.05</td>
</tr>
<tr>
<td>Survival rate (%)</td>
<td>96.00 ± 0.25</td>
<td>97.00 ± 0.52</td>
<td>98.00 ± 0.75</td>
</tr>
</tbody>
</table>

1 Standard Deviation; 2 Different superscripts within a row indicate significant differences at p < 0.05
The mean (± SD) of lactic acid bacteria (lab) count (CFU/g) to total autochthonous bacteria count (CFU/g) ratio (%) of intestine extracted from juvenile Khaju fish fed the experimental diets after 63 days at three replicates. Different letters indicate significant differences (p < 0.05).

**Results**

**Growth performance and survival rate**

There were no significant differences in the initial weight of treatment groups (p > 0.05). Increasing the levels of dietary FOS from 5 to 30 g/kg had significant (p < 0.05) effects on the final weight and specific growth rate (SGR) of juvenile Khaju fish compared with control diet (Table 2; Figure 1). VFI (2.06-3.12 % BW/day) and survival rate (97-99%) of juvenile Khaju fish fed the diets containing 5-30 g FOS kg⁻¹ were significantly (p<0.05) higher than those of fed the control diet (1.12 % BW/day and 96%; respectively) (Table 2). Based on the broken line regression model, the dietary FOS re-

**Figure 4**

Table 3

The mean (± SD) of haematological parameters and differential leucocyte counts of juvenile Khaju fish fed the experimental diets after 63 days (n=3)²

<table>
<thead>
<tr>
<th>Control</th>
<th>Dietary fructo-oligosaccharide (g/kg)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>5</td>
<td>10</td>
</tr>
<tr>
<td>Erythrocyte count (x10⁶/µl)</td>
<td>1.87 ± 0.07</td>
<td>1.89 ± 0.80</td>
</tr>
<tr>
<td>Hb (mmol/l)</td>
<td>3.01 ± 0.11 a</td>
<td>3.05 ± 0.13 ab</td>
</tr>
<tr>
<td>Haematocrit (%)</td>
<td>24.10 ± 0.13 a</td>
<td>24.17 ± 0.14 a</td>
</tr>
<tr>
<td>MCV (fl)</td>
<td>151.28 ± 1.23 a</td>
<td>152.00 ± 1.33 a</td>
</tr>
<tr>
<td>MCH (pg)</td>
<td>32.16 ± 1.11 a</td>
<td>33.25 ± 1.13 ab</td>
</tr>
<tr>
<td>MCHC (mmol/l)</td>
<td>13.68 ± 1.34</td>
<td>13.72 ± 1.34</td>
</tr>
<tr>
<td>Leucocyte count (x10³/µl)</td>
<td>34.45 ± 1.39</td>
<td>35.15 ± 1.27</td>
</tr>
<tr>
<td>Lymphocytes (%)</td>
<td>87.13 ± 1.30 a</td>
<td>88.27 ± 1.45 ab</td>
</tr>
<tr>
<td>Neutrophils (%)</td>
<td>5.21 ± 1.23</td>
<td>5.89 ± 1.56</td>
</tr>
<tr>
<td>Monocytes (%)</td>
<td>7.66 ± 1.53 b</td>
<td>5.84 ± 1.01 a</td>
</tr>
</tbody>
</table>

1 Standard Deviation; 2 Different superscripts within a row indicate significant differences at p < 0.05;
requirement for maximum growth (SGR) and minimum FCR of juvenile Khaju fish were estimated to be 18.18 and 18.29 g/kg, respectively (Figure 1).

**Nutritional efficiency indices (NEIs)**

The juvenile Khaju fish fed the control diet showed significantly \((p < 0.05)\) lower values for NEIs (Figure 2). Increasing the levels of dietary FOS from 5 to 30 g/kg had significant \((p < 0.05)\) effects on PER (1.56-2.12) and PPV (48.76-52.92\%) compared with fed control diet (1.13 and 40.23\%, respectively). Based on the broken line regression model, the dietary FOS requirement for maximum PER and PPV of juvenile Khaju fish were estimated to be 23.79 and 22.27 \%, respectively (Figure 2).

**Haemato-immunological parameters**

The effects of dietary FOS supplementation on the haemato-immunological parameters of juvenile Khaju fish are summarized in Table 3 and Figure 3. Statistical analyses revealed that erythrocyte count, MCHC, leucocyte count and neutrophils were not significantly affected by dietary FOS supplementation \((p > 0.05)\); Table 3). Hb, haematocrit, MCV, MCH and lymphocyte of the juvenile Khaju fish fed the diets containing 20 and 30 g/kg FOS were significantly \((p < 0.05)\) higher than those of control diet (Table 3). After a 63-day feeding trial, dietary FOS supplementation (10-30 g/kg) significantly \((p < 0.05)\) increased the activities of IG and ACP compared with those fed with 10 g/kg FOS and control diet (Figures 3a, 3c). Feeding 5-30 g/kg FOS in Kha-ju fish significantly \((p < 0.05)\) increased the LYZ activity compared with control group (Figure 3b). After 5 min air exposure challenge, the significant-

**Digestive enzyme activities**

Amylase activity (0.37-0.45 U/mg protein/min) was not significantly affected by dietary FOS supplementation (Table 4). Trypsin activity of fish fed 5-30 g/kg FOS were significantly \((p < 0.05)\) higher than that of control treatment (2.30-4.03 vs. 1.87 U/mg protein/min) (Table 4). The significantly \((p < 0.05)\) higher trypsin activity (4.03 U/mg protein/min) was observed in fish fed with 20 g/kg FOS (Table 4). Lipase activity in fish fed 10-30 g/kg FOS (2.25-2.95 U/mg protein/min) showed significantly higher activity than those fed the diet 5 g/kg FOS and control group (Table 4).

**Microbiological analysis**

The lab count/total autochthonous bacteria count ratio in the extracted intestine in the juvenile Khaju fish fed the diets containing 5-30 g/kg FOS (34.5-79\%) showed significantly \((p < 0.05)\) higher values than those fed control diet (23.3\%) (Figure 4). The significantly higher \((p < 0.05)\) lab count/total autochthonous bacteria count ratio was observed in Khaju fish fed the diet containing 20 g/kg FOS (Figure 4).

**Discussion**

**Growth performance, survival rate and digestive enzymes**

The main challenge facing Khaju fish culture as a domesticated species in the northeast of Iran is the improvement of feed formulation in or-

| Table 4 | The mean (± SD\(^1\)) of initial weight (g), final weight (g), voluntary feed intake (% BW/day) and survival rate (%) of juvenile Khaju fish fed the experimental diets after 63 days (n=3)\(^2\) |
|---------------------------|---------------------------|---------------------------|---------------------------|
| **Dietary fructo-oligosaccharide (g/kg)** | **Control** | **5** | **10** | **20** | **30** | **p value** |
| Trypsin (U/mg protein/min) | 1.87 ± 0.15\(^4\) | 2.30 ± 0.11\(^b\) | 3.11 ± 0.13\(^c\) | 4.03 ± 0.21\(^d\) | 3.20 ± 0.20\(^e\) | 0.0001 |
| Lipase (U/mg protein/min) | 1.62 ± 0.21\(^a\) | 2.12 ± 0.31\(^b\) | 2.25 ± 0.92\(^c\) | 2.95 ± 0.18\(^b\) | 2.73 ± 0.31\(^b\) | 0.001 |
| Amylase (U/mg protein/min) | 0.38 ± 0.01 | 0.37 ± 0.02 | 0.41 ± 0.07 | 0.45 ± 0.09 | 0.43 ± 0.08 | 0.452 |

\(^1\) Standard Deviation; \(^2\) Different superscripts within a row indicate significant differences at \(p < 0.05\)
der to optimize growth performance and disease resistance through the development of health-promoting diets [23,24]. Critically evaluation of key parameters such as feeding regime (feedstuffs, inclusion levels and supplements) and preference in cultivable aquatic species (behavioral aspects and palatability) helps aqua-feed industry to formulate suitable diets according to biological requirements [6,8]. To our knowledge, this is the first study to investigate the effects of dietary FOS supplementation on growth performance, haemato-immunological parameters, nutritional efficiency indices, cultivable gut microbiota, digestive enzymes and stress resistance of juvenile Khaju fish. The results of present study showed that 5-30 g/kg FOS had significant effects on growth performance of Khaju fish. These results are according to findings in blunt snout bream (Megalobrama amblycephala) [20], stellate sturgeon (Acipenser stellatus) [19], rainbow trout (Oncorhynchus mykiss) [25] and red drum (Sciaenops ocellatus) [22]. However, FOS supplementation in the diets of carp (Cypri- nus carpio) fry [10], beluga (Huso huso) juvenile [21] and Atlantic salmon (Salmo salar) [26] had no significant effects on growth performance. Using prebiotics as a feed additive is considered as a way to maximize the potential of modulating the mucosa-associated microbiota and luminal bifidobacteria to efficiently discriminate and eliminate pathogenic organisms [7,27]. Consequently, the developed gastrointestinal microflora balance leads to boost In vivo digestion and absorption processes and finally, improvement of host health and growth performance [7,28,29]. Overall improvement of FCR may be regarded to produce some metabolites such as butyrate in the gastro-intestinal tract [22]. A general increase in propionate and total fatty acid production was recorded through In vitro incubation of red drum, Sciaenops ocellatus chyme with Grobiotic® in the culture media [30]. The differences observed in the results of these studies might be due to the prebiotic chemical properties (origin and DP), feeding trial design (initial weight, feeding period, food formulation etc) and tested biological indices [7,8,13]. Improvements in the nutrient retention efficiency in the aquatic species are needed to reduce the environmental impacts of aquaculture, also, to make more efficient use of dietary nutrients. Perhaps the greatest potential for improving nutrient retention lies in the selection of broodstock having higher nutrient retention rates, but opportunities to im-

prove protein retention through the formulation of ideal proteins and by increasing dietary energy levels or supplements influencing metabolism pathways also needed to be explored [7,31].

Dietary FOS supplementation significantly increased the survival rate of juvenile Khaju fish compared with the control group. Similarly, improved survival of common carp fry [10], cobia [Rachycentron canadum] larvae [32], rainbow trout [33] and beluga juveniles [21] has been reported upon FOS prebiotic administration. This can be related to improved general health or immune status [7,18].

Data from the present study can confirm that FOS as a non-digestible feed ingredient via selective fermentation affected the composition of intestinal microflora by stimulating Bifidobacteria and Lactobacillilli, which are present in the intestine bacterial flora. Burr et al. reported the supplementation of soybean meal-based diets of red drum with mannanoligosaccharide, trans-galactooligosaccharide and Grobiotic (a mixture of partially autolysed brewer’s yeast, dairy components and dried fermentation products) increased apparent digestibility coefficient (ADC) of nutrients than those of control diet [30]. Improvement of ADC values could be potentially related to up-regulation of the activities of specific digestive enzymes [22]. In the present study, the activities of digestive enzymes including trypsin and lipase in juvenile Khaju fish fed the diets containing FOS were higher than those of control. In this regard, supplemented-diets containing FOS significantly increased the activities of amylase and total protease in male broilers [34]. The effect of dietary-supplementation of different prebiotics was obvious after 49 days in chicken and 7 weeks or more in fish [22] because the development of microbial ecology and synchronous changes in the morphology and function of GIT are very complex processes that depends on several factors including the enteric environment, host physiology, microbial interactions, nutrition history and genetics [6,35,36].

Haemato-immunological parameters and stress resistance

Haemato-immunological parameters are considered as valuable tools in order to evaluate the potential of prebiotics in aquafeeds [14,37,38]. The results of the present study showed that dietary FOS supplementation had significant effects

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on Hb, haematocrit, MCV, MCH, lymphocytes and monocytes. Similar results were observed on stellate sturgeon [19] and beluga [21]. In the present study, dietary FOS supplementation (10-30 g/kg) increased the IG, LYZ and ACP activities compared with the control treatment. Similarly, symbiotic administration in large yellow croaker (Larimichthys crocea) [39], gilthead sea bream (Sparus aurata L.) [40], koi (Cyprinus carpio koi) [41] and common carp fry [10] revealed a significantly higher respiratory burst activity. Elevation of lymphocytes, IG, LYZ and ACP activities is probably caused by the stimulation of Khaju fish immune responses due to FOS supplementation.

The intestinal flora of fish as a dynamic environment containing a diverse community of micro-organism [42,43] was affected by genetic, nutritional and environmental factors [44]. In spite of considering minor ratio of LAB count in intestinal microbiota, they have been regarded as beneficial components of the fish intestine [45]. Dietary manipulations using probiotics and prebiotics can increase the resistance of animals through mechanism of pathogen inhibition in GIT [46]. Competition for territory in GIT, reduction in pH and release of natural antibiotics from beneficial microbial populations can cause the pathogen inhibition [47]. Enhanced resistance also might be attributable to the beneficial effect of GIT microbes on host innate and adaptive immunity [48]. In this regard, lactic acid bacteria-containing yogurt could inhibit the growth of intestinal carcinoma through increased activity of immunoglobulin A, T cells and macrophages in mice [49]. In the present study, dietary FOS of 10-30 g/kg supplementation significantly increased the ratio of total authochthonous intestinal heterotrophic bacteria count to LAB count. The changes in the ratios of lactocabacillus count/total count noted in the present study may have benefited the Khaju fish, possibly by increasing nonspecific immune responses and by increasing concentrations and/or production of volatile fatty acids and butyrate as by-products of fermentation process in GIT. Both of these benefits have been demonstrated in chicken [50] and swine [51]. It was confirmed that butyrate as an increasing factor of disease resistance could down-regulate the expression of invasion genes in Salmonella sp. [52], although, the values of volatile fatty acids and butyrate were not measured in this study. However, further evaluations with use of denaturing gradient gel electrophoresis [DGGE] and 16S rRNA sequencing need to identify the LAB species in order to confirm their beneficial effects in juvenile Khaju fish. Similar to the present results, FOS has been reported to increase the levels of total cultivable autochthonous intestinal heterotrophic bacteria and LAB in common carp fry [10], stellate sturgeon [19] and beluga juveniles [21]. Although, the results of the present study confirmed that modulation of the intestinal microbiota in Khaju fish can be achieved through dietary FOS supplementation, but further considerations are needed.

Resistance during different stressors (physical, chemical and biological) has been considered as an important indicator to evaluate the efficiency of feed [7,53]. The critical factors to set up a challenge test are type, quantity and exposure time of stressor, genetic and feeding history of target animal, sampling method (plasma or serum) and physicochemical conditions of rearing facilities [7,54]. Regarding the focus of aquaculture industry to increase stocking rate, air exposure challenge is considered as one of limiting factors of production. It has been confirmed that numbers of activated macrophages (innate immunity), T-cell (adaptive immunity) activation and the recruitment of surveillance T cells increased during an acute stress as a primary stress response [55,56]. The results of the present study revealed that dietary FOS supplementation significantly increased fish resistance in the air exposure challenge test compared with the control diet and this is in accordance with previous reports on pacu (Piaractus brachypomous) [57], silver dollar (Metynnis argenteus) [58] and zebra fish (Danio rerio) [59] fed dietary FOS supplementation. Although, it is not clear how to describe the results of previous studies, it can be related to up-regulate immune system and finally, to obtain a higher level of homeostasis via the metabolic pathways of oxygen consumption process by mitochondria [7,24,31].

**Conclusion**

In the current trial, dietary FOS supplementation had significant effects on the growth performance and haemato-immunological parameters of juvenile Khaju fish and modulated autochthonous gut microbiota levels and stress resistance. The results of this study boost planning further studies on the use of FOS and other prebiotics in juvenile Khaju fish. To distinguish the metabolic pathways...
and optimum inclusion level is a topic that guides further research.

Materials and methods

Experimental diets

A basal diet (350 g/kg, crude protein; 120 g/kg, crude fat; 18.9 MJ/kg, Gross energy) as control diet (Table 1) was formulated [NRC, 2011; Safari, 2016] with WUFFDA (windows-based user-friendly feed formulation, done again; University of Georgia, Georgia, USA) software. The prebiotic, fructooligosaccharide (FOS, Raftilose® P95, Orafti Co., Belgium) was used at four doses 5, 10, 20 and 30 g/kg in the place of carboxymethyl cellulose [14]. The minimum level of fructose in FOS guaranteed by manufacturer is 91%. DP of fructose in FOS ranges from 2-8% (Mahious, A. S., Ollevier, 2005). The other components are mainly glucose, fructose and sucrose. All feedstuffs were ground to a particle size < 250 μm [Glencross et al., 2007]. After adding fish oil, supplements and water (320 g/kg) contents, respectively, the mash was transferred from the hand pelletizer (Abzarsazan CO, Iran) with a 3 mm die, dried at 30°C, packed in three-layer water-proof nylon bags and maintained at -20°C [Hardy and Barrows, 2003] until feeding trials were started.

Fish culture and feeding trial

Two hundred twenty five healthy juvenile Khaju fish (68.5 ± 1.52 g) were obtained from the Zahak reproduction and restocking center of warmwater and native fishes (Zabol, Iran) and stocked at a density of ten fish per 100 L tank (0.8 × 0.25 × 0.5 m) in a semi-recirculating system with daily water exchange rate of 30% at three replicates for each experimental diet. Most adapted to the feeding regime within three days. Unconsumed feed was collected three hours after feeding and weighed. Water temperature was maintained at 22.9°C throughout the feeding trial. DO (5.3 ± 0.10 mg/l), pH (8.6 ± 0.15), hardness (115 ± 1.3 mg/l as CaCO₃), unionized ammonia (< 0.06 mg/l) and nitrite contents (< 0.6 mg/l) were evaluated every week. Animals were held under L:D 12:12 h. Each diet was randomly assigned to three tanks of Khaju fish and they were fed 4% body weight twice daily (6:00 and 14:00) for 63 days. Biometry was done during first and last day of the experiment.

Evaluation of growth performance, survival rate and nutritional efficiency indices

At the end of the feeding trial, each fish was individually weighed (± 0.01) on an electronic scale (AND, Japan). All parameters were corrected based on the ingested feed. Growth parameters, survival rate and nutrient efficiency indices (PER and PPV) were calculated as follows [7]:

\[
\text{Specific Growth Rate (SGR ; %/day)} = \left(\frac{\ln W_f - \ln W_i}{t}\right) \times 100
\]

\[
\text{Voluntary Feed Intake (VFI; % body weight/day)} = \left(\frac{\text{Feed consumed (DM)}}{\text{Wmean } \times t}\right)
\]

\[
\text{Survival Rate ( %)} = \left(\frac{\text{Final Individual Numbers} / \text{Initial Individual Numbers}}{100}\right)
\]

\[
\text{Feed Conversion Ratio (FCR)} = \left(\frac{\text{Feed consumed}}{\text{W gain}}\right)
\]

Protein Efficiency Ratio (PER) = \(\frac{\text{Wgain}}{\text{Crude protein consumed}}\)

Protein Productive Value (PPV; %) = \(100 \times \left(\frac{\text{Protein retained}}{\text{Protein consumed}}\right)\)

In the above equations, \(Wi\), \(Wf\), \(Wmean\) and \(Wgain\), \(t\), and \(\text{Feed consumed}\) are initial weight, final weight, mean weight, weight increment (g), time period (day) and consumed feed (g), respectively.

Haemato-immunological analysis

Six fish from each tank (18 fish per treatment) were anaesthetized by clove solution after 24 h of last feeding time in the 63th day. About 3.5 ml of blood was drawn from the caudal vein. Then, blood samples were introduced to both heparinized and non-heparinized tubes to perform hematological and immunological studies, respectively. For serum isolation, blood samples in nonheparinized tubes were centrifugated at 1000g for 5 min in order to separate the plasma. All assays were done one by one at three replicates. Whole blood was suspended in the diluents described by Natt and Herrick in order to count erythrocyte cells (RBC) and total leucocyte cells (WBC) [60]. Haematocrit (Ht) was determined using the micro-Ht method as described by Brown and Ht values are reported as packed cell volume percentage [61]. Mean corpuscular hemoglobin content (MCV) and hemoglobin (Hb) was estimated using Sahlı’s method according to method explained by Baxhall and Daley [62]. Differential WBC (neutrophils, lymphocytes and monocytes) were done using May-Grunwald-Giemsa blood smears. The non-heparinized tubes were stored at 20°C to measure the activities of total immunoglobulin (IG), lysozyme (LYZ) and alternative complement (ACP) (24).

Digestive enzyme activities

At day 63th, three fish (9 fish per treatment) were starved for 24 h and sampled from each tank for enzymatic analysis. The intestines were isolated and rinsed with cold distilled water at 4°C [24]. The intestinal enzyme extracts were homogenized in phosphate buffered saline (pH 7.5; 30 g/70 ml PBS) using a homogenizer (DI 18 Disperser) and the homogenate was then centrifuged at 15000×g, 4°C, 15 min and the supernatant stored in liquid nitrogen until further analysis. The total protein content of the supernatant was determined as using bovine serum albumin as a standard [24]. Protease activity was measured using casein hydrolysis at pH 8 [24]. Amylase activity was quantified using starch as a substrate at 540 nm [24] and lipase activity was determined via the measurement of fatty acids released following enzymatic hydrolysis of triglycerides in a stabilized emulsion of olive oil [24]. Digestive enzyme activities (i.e. protease, amylase and lipase) were defined as specific activity (U/mg protein/min).

Chemical analysis

Analysis of dry matter (oven drying, 105°C), crude protein (N × 6.25, Kjeldahl system: Buchi Labortecnik AG, Flawil, Switzerland), crude fat (Soxtec System HT 1043; Foss Tecator, AB), ash (muffle furnace, 550°C), gross energy (Parr bomb calorimeter model 1266, Parr Instrument Co., Moline, IL) and crude fiber (after digestion with H2SO4 and NaOH) analysis of feedstuffs, diets and feces were performed according to standard methods [7]. Nitrogen free extract (NFE) was cal-

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culated by subtraction of dry matter from crude protein, crude fat, crude fiber and ash contents.

**Microbiological analysis**

To quantify total viable autochthonous heterotrophic aerobic bacteria and lactic acid bacteria (LAB), six fish per treatment were transported alive to laboratory, anesthetized with ice, rinsed with benzalkonium chloride (0.1% for 60 min) and dissected with scalpels without pooling samples. Then, entire intestine was removed, homogenized with sodium chloride (0.9 w/v) using a homogenizer (DI 18 Disperser) and the homogenate was then centrifuged at 5000×g, 4°C, for 5 min. Serial dilutions 10⁻¹⁻¹⁰⁻⁷ were prepared. Nutrient agar (Sigma-Aldrich Co.) and MRS (Merk Co.) were used to determine total viable autochthonous heterotrophic aerobic bacteria and lactic acid bacteria count at room temperature (25°C) for 5 days, respectively. Colony-forming unit (CFU) per gram was calculated from statistically viable plates (i.e. plates containing 30 to 300 colonies) [7, 21].

**Air exposure challenge**

Three fishes from each tank (9 individuals per a treatment) were exposed to air for 5 min on day 63 [24]. The activities of IG, LYZ and ACP were used to evaluate biological responses of fish fed the experimental diets against air exposure challenge.

**Statistical analysis**

All percentage data were transformed using arcsine method. After confirming the homogeneity of variance and normality of the data using Leaven and Kolmogorov-Smirnov tests, one-way ANOVA was used to compare the treatments at three replicates. Duncan test was applied to compare significant differences among treatments (p < 0.05) with SPSS version 19. All results were given as mean ± SD.

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**Author Contributions**

Designed the experiment: O.S.; Performed the experiment: F.S.; Analyzed the results: R.V.

**Conflict of Interest**

None of the authors of this paper have a financial or personal relationship with other people or organizations that could inappropriately influence or bias the content of the paper.

**References**


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24. Safari O. Study on the possibility of optimum diet production of snow trouts (Schizothorax pelzami and Schizothorax zarudnyi) as native species in Iran with aim of sustainable production in the aquaculture systems. FUM. Project no: 33036. Mashhad, Iran; 2016.


57. Nikparvar E. Study on the effect of different levels of mannan-oligosaccharide on some biological indices of juvenile pacu (Piaractus brachypomus). Chabahar Maritime University; 2016.

58. Ahmadi I. Study on the effect of different levels of mannan-oligosaccharide on some biological indices of juvenile silver dollar (Metynnis argenteus). FUM. Project no: 23245. Mashhad, Iran; 2015.

59. Soleimani A. Study on the effect of different levels of mannan-oligosaccharide on some biological indices of juvenile zebra fish (Danio rerio). Poultry Sci;1952.31:735-738.


Sensory evaluation of the color of mutton by computer vision system

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Keywords
Sheep, Meat color, Sensory evaluation, Mutton

Abstract
Evaluation of meat color by a computer vision system (CVS) is a promising implement to dominate the difficulties when the meat is directly evaluated. In this study, 60 Longissimus dorsi from different carcasses of sheep were provided and cut into samples in 5 mm thickness. Immediately under standard shooting conditions, photographing was carried out by CVS. At the same time, the color of meat was measured with Hunterlab colorimeter. The first photo was taken on samples on a freshly cut surface just arrived at the laboratory and the others on 3rd, 5th, 7th, 9th, 11th, and 13th days after slaughtering. Then, seven trained sensory panels were asked to evaluate the color of the photos that were taken during 13 days and graded them in order of preference. In general sensory panel preferred samples with high lightness, a relatively high redness, and yellowness until 7 days after slaughtering.

Abbreviations
CVS: Computer Vision System
WHC: Water Holding Capacity
ISO: International Standard Organization
LED: Light-Emitting Diode
CMOS: Complementary Metal-Oxide Semiconductor sensor
AMSA: American Meat Science Association
RGB: Red, Green and Blue
HSI: Hue, Saturation and Intensity
**Introduction**

Meat is an essential component of the diet of human kind and its consumption is affected by various factors. The most important factors affecting consumption are product characteristics (sensory and nutritional properties, price, safety, convenience, etc.), and consumer and environment related characteristics such as health, psychological, climate, family or educational aspects, general economic situation, legislation [1].

Lean quality in fresh meat refers to numerous factors, but predominantly focuses on muscle pH, water holding capacity (WHC) and color. These factors are the main quality attributes that affect directly the raw product attractiveness to potential customers and influence technological properties for processed products [2, 3]. Among these factors, color of meat is the most important characteristics for the consumer. This is because the visual feeling is the first sensation of most foods, so it plays a significant role in consumer decision [4-6]. Mostly, the consumer’s willingness to use a food depends on the appearance that depends to the shape, structure, color, quality and the relationship with the surrounding context observed through the eyes. Appearance by itself can affect expectations about other organoleptic characteristics as well [7, 8]. In the case of meat, color is one of the most indispensable organoleptic characteristics. It influences the acceptability of the product and plays a significant role in the purchasing decisions [4-6, 9].

There are several methods to measure the color of meat, like the visual appraisal and instrumental analysis using spectrophotometer and colorimeter [10, 11]. Computer vision system (CVS) is another method for measuring the color of meat. All of these instruments are fast, accurate and easy to apply, but they don't give a measure of consumer preference since they express data as color space coordinates.

Visual appraisal is the meat color assessment most closely related to consumer evaluation. However, it is time-consuming, complex and expensive. The long period for the sensory evaluation negatively affects the meat color stability and acceptability [12]. Difficulties related to the color evaluation of meat by consumer surveys are resolved through the evaluation of images [13-15]. Therefore, this study will provide additional information regarding the role of color as it relates to the quality of fresh meat eating.

The purpose of this study was to evaluate the possibility of using images and CVS for the sensory evaluation of mutton color, to document the effect of meat color on fresh lamb consumption acceptance and develop a descriptive analysis profile for uncooked mutton chops of different color classifications.

<table>
<thead>
<tr>
<th>Method</th>
<th>Sample</th>
<th>Days</th>
<th>L*</th>
<th>a*</th>
<th>b*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Mean ± SD</td>
<td>Mean ± SD</td>
<td>Mean ± SD</td>
</tr>
<tr>
<td>CVS</td>
<td>A</td>
<td>1</td>
<td>41.3 ± 1.86</td>
<td>14.46 ± 0.1</td>
<td>11.9 ± 0.05</td>
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<tr>
<td></td>
<td>B</td>
<td>3</td>
<td>41.7 ± 0.11</td>
<td>13.71 ± 0.06</td>
<td>12.02 ± 0.1</td>
</tr>
<tr>
<td></td>
<td>C</td>
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<tr>
<td></td>
<td>D</td>
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<td>42.18 ± 1.55</td>
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<td></td>
<td>E</td>
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<td>40.9 ± 0.62</td>
<td>13.68 ± 0.1</td>
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<tr>
<td></td>
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<td>13.98 ± 0.05</td>
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<tr>
<td></td>
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<td>14.23 ± 0.02</td>
<td>10.41 ± 0.36</td>
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<tr>
<td>Colorimeter</td>
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<td>17.33 ± 0.18</td>
<td>11.54 ± 0.12</td>
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<td>12.02 ± 0.05</td>
<td>12.14 ± 0.05</td>
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<tr>
<td></td>
<td>C</td>
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<tr>
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<td></td>
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<td>13.25 ± 0.1</td>
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</tr>
<tr>
<td></td>
<td>G</td>
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<td>32.91 ± 0.79</td>
<td>14.85 ± 0.05</td>
<td>9.36 ± 0.57</td>
</tr>
</tbody>
</table>
Results

Table 1 illustrates the obtained results from high lightness ($L^*$), a relatively high redness ($a^*$), and yellowness ($b^*$) indexes throughout 13 days of meat storage in the refrigerator using colorimetric and CVS methods. The changes of these indexes throughout 13 days storage at refrigerator have also been shown in Figure 1 (a, b and c). The quantities of $L^*$ and $b^*$ values in photos, measured by two devices, the colorimeter and CVS, increased until the seventh day and then decreased. On the contrary, the amount of $a^*$ value decreased until the seventh day and then increased and a slight reduction of the $a^*/b^*$ ratio was observed.

An acceptable preference was noticed between the images of samples until the seventh day and day seven afterward. With regard to this finding, it should be noted that the samples remained in storage at the same time temperature, which could have caused the surfaces discoloration. On the previous days, the consumers could not distinguish the samples properly, which led to the presence of the three forms of myoglobin on the meat surface. According to the images, samples A, B, C and D were preferred to the other samples ($p < 0.05$). In addition, the sample G was labeled the least proper sample ($p < 0.05$).

Consumers were able to distinguish the samples of the 1st, 3rd, 5th and 7th days from those belonging to the 9th, 11th and 13th day. Therefore, the color assessment could be more accurate if carried out on a freshly cut surface and after 7 days of keeping the samples in the refrigerator.

The instrumental color measurements of the seven samples are reported in Table 1. It is not straightforward to compare the results of the preference test with those of the instrumental analysis and to find out the relationships between a single preference decision with the trichromatic proportions which are linked to each other in different ways. The panels did not approve of many samples such as samples F and G, due to low lightness. As can be seen in image G, the highest $a^*/b^*$ ratio in sample G was indicative of the deep, dark red color of the meat. However the consumers preferred sample A, which had a bright red color, with the highest lightness and relatively high yellowness. Samples B, C and D which had the same $a^*$ and $b^*$ values as sample A, were also assessed at the lowest lightness.

According to Figure 2, the images of the meat samples which were kept in the refrigerator, were considered acceptable by the consumers until day seven (line 5 shows the acceptable level). As mentioned earlier, the lightness of the surface of meat increased until day seven and reduced afterwards, denoting the freshness of meat in the viewpoint of the observers.

Discussion

Computer vision (CV) system has been widely used for measuring color, fat, and other physical characteristics of meat. Studies have been reported developments of novel hardware for machine vision systems and software algorithms for image processing to extract useful information for rapid and non-destructive detection of physical quality attributes of meat. Chen et al., (2010) used a com-
puter vision system and a color digital camera to measure the fat color of beef for quality grading [16]. Girolami et al. (2013) utilized two instruments as Minolta CR-400 chronometer and CVS in order to investigate the color of beef, pork, and chicken meat. They compared these two methods to traditional method of meat quality determination by panelists. Therefore, by using three tests, the panelists realized the similarity of the digital images to the actual samples (p < 0.001). The results of this study revealed the fact that CVS illustrated more realistic colors than colorimeter [8].

Sun et al., (2014) reported extraction of color features (means and standard deviations) in terms of RGB (Red, Green and Blue) and HSI (hue, saturation, and intensity). The color in the sample images was correlated to the moisture content obtained by chemical analysis, which showed encouraging correlation coefficients of 0.56 (for models built by PLSR) and 0.45 (for models built by a neural network algorithm). The study showed a potential for machine vision and image processing for detection of chemical contents in meat. Research on the relationship of color to chemical, physical, and biological changes can enhance the accuracy of image processing for rapid and non-destructive detection of meat quality attributes. Application of a digital camera with an auxiliary lighting system was reported for prediction of troponin-T degradation in beef Longissimus dorsi using texture features from color images [17].

The results of this study indicate that the lighting surface of meat increased until the seventh day and then reduced. This issue illustrates the freshness of meat through the vision of beholder. According to chemical reactions taken place in fresh meat, the color of sliced meat is reddish-purple. This color can be observed in the parts not having reached any oxygen. After contacting the meat with the air, its surface turns red. Moreover the surface of the meat increased by 2-3 millimeters. After keeping the red meat for 1-3 days at 2-40°C, one brownish layer of metmyoglobin is formed in the deepest layer of oxymyoglobin. Metmyoglobin is formed quickly and this is due to its high capability of absorbing oxygen from deoxymyoglobin and oxymyoglobin. After some days keeping, oxymyoglobin thickness reduces and correspondingly metmyoglobin thickness increases. This issue was observed to diminish the lighting of the surface of meat during preservation [18].

Leon et al. (2006) presented a precise method for extracting L*, a*, and b* indices from RGB im-

![Figure 3](Image)

**Figure 3**
Computer vision system
Having been slaughtered, samples were provided in approximately mutton samples. The age of the sheep was a year and around.

Preparation of samples

**Materials and methods**

The validated parameters.

the color correctness of the image and checking to obtain true reproduction of the meat. For this purpose, camera setting, lighting and background are very important to standardize shooting conditions over a long time, with the ability to collect a large number of ratings from many consumers. However, it is very important to standardize shooting conditions such as camera setting, lighting and background to obtain true reproduction of the meat. For this purpose, a color chart is a useful tool for adjusting the color correctness of the image and checking the validated parameters.

Although computer vision system and image analysis have proven useful for quantifying the quality of different meat and meat products, little research has been reported on the measurement of lamb quality attributes. Chandraratne et al., (2006), predicted the cooked lamb tenderness by geometric and texture analyses of images obtained from a color digital camera. Also, they used a machine vision system consisting of three CCD digital color cameras for grading lamb carcasses [24].

In conclusion, when the meat is directly evaluated, using the sensory evaluation of meat color by computer vision system is a convenient tool to overcome the difficulties. Images can be used for a long time, with the ability to collect a large number of ratings from many consumers. However, it is very important to standardize shooting conditions such as camera setting, lighting and background to obtain true reproduction of the meat. For this purpose, a color chart is a useful tool for adjusting the color correctness of the image and checking the validated parameters.

**Materials and methods**

**Preparation of samples**

This study was carried out on 60 Longissimus dorsi of mutton samples. The age of the sheep was a year and around. Having been slaughtered, samples were provided in approximately 24 hours. Before analyzing color, samples were divided into similar slices with 5mm thickness using the cutter. Then, the samples’ superficial moisture was removed by a cloth and squeezed to reduce surface reflection [25]. In order to evaluate the color of the meat through three evaluation methods, the meat piece was kept in the refrigerator for 13 days and imaged in the 1st, 3rd, 5th, 9th, 11th and 13th days after the analysis of the images, the color indices were used for further assessment. Simultaneously, the meat color indices were obtained through a colorimetric device. Furthermore, color sensory evaluation was performed, as explained in Table 2.

**Computer vision system**

The CVS for taking photos in this study was a Nikon (Coolpix P510, Japan) digital camera with complementary metal-oxide semiconductor sensor (CMOS) which located at the distance of 20cm vertically from the sample. Camera settings were as follows: exposure: f/4: 0, sensor sensitivity to light: 400, camera flash: deactivated or switched off, shutter speed: 1.50 frame per second, sensitivity to fluorescent light: activated or turned on, camera zoom: none, focal length: 33mm and image resolution 4608*3456 pixels. The light-emitting diode (LED) arrays were settled at 20cm distance from the sample inside and over ceiling wooden box. Also, the camera was settled outside and over a wooden box covered with black opaque sheets. In order to minimize reflection, black opaque sheets were substantial. The CVS in this study is shown in Figure 3.

**Color assessment**

In this study, the color of the samples was evaluated using the Hunterlab instrument (45/0, CX2547, USA). The intended instrument reveals the color of food in solution and solid states through three indices as L*, a*, and b*. This device includes one circular glassy cell with limited beam in which food materials are placed into it and reveals the average color indices for its covering domain. Therefore, this method would not be suitable especially for foods, excluding homogeneous color changes.

**Sensory analysis**

Selection of panelists was carried out according to a method previously described by Girolami et al. (2013). This selection was conducted by applying the Ishihara tables (26) to identify possible visual abnormalities such as color-blindness in the red spectrum. After selecting the panel, the candidates with normal vision were also subjected to a triangle test conducted in accordance with international standard 4120/2004 (ISO 2004), to determine the odd one among 3 colored samples. The minimum passing score was 8 out of 10.

Sensory assessment was performed by using nine-point hedonic scale (1 for unacceptable and 9 for acceptable color of meat) according to American Meat Science Association (AMSA) instruction (18) (Table 2) and CIE17 international standard [16]. 7 referees were selected for this research, belonging to the production and meat quality control sections of Mashhad, and they were all familiar with meat characteristics. After slaughtering, the provided images from the sliced mutton with 5mm diameter were at the referees’ disposal in the 1st, 3rd, 5th, 9th, 11th, and 13th days and tellingly were asked to score each image from 1 to 9.

According to the CIE17 standard [16], observations were carried out in a suitable place under the light controlled conditions (type, amount, and direction), the environmental cir-
cumstance, and geometric conditions such as the relative light source position, sample, and the eye. In this experiment, artificial light (fluorescent light) was provided in a room, which was composed of white walls and floors, in order to prevent the reciprocal interactions or color adjustments, while also inhibiting the effect of light reflecting for accurate assessment of the panelists’ viewpoints.

As far as changes in the lighting, sample, eye position of the panellist, influence the obtained results, therefore; geometrical condition should be standardized.

Furthermore, the taken images from the sample were placed in one stable spot of the room and the panelists were asked to enter the room separately to evaluate the images. For minimizing the direct reflection of the light from the surface, the angle between the panellists’ eye-tracking and the surface in which the samples were placed had to be different from the angle at which the light from the light source meets the surface. In this direction, the images were placed in such a way that they made 45 degrees with panellist’s vision. Also, the lighting resource was placed over the ceiling and shined vertically over the sample.

**Statistical analysis**

The evaluation of this test was conducted through IBM SPSS Software (Version 20.0). The Student’s t-test was used to locate differences between colorimeter and CVS measurements. P-values of 0.05 or less were considered significant. The correlation coefficient between CVS and colorimeter measures was evaluated using the Spearman rank correlation test. Friedman’s test was used to evaluate the average rates of assigned to the images by the panellists during the 13-day period.

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**Author Contributions**

All authors contributed to the design of study, data analysis and manuscript preparation.

**Conflict of Interest**

The authors declare that there is no conflict of interest.

**References**


NetB negative *Clostridium perfringens* infection associated with acute necrotic enteritis in mynah (*Acridotheres tristis*), grey partridge (*Perdix perdix*) and turkey (*Meleagris gallopavo*)

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**Keywords**

Mynah (*Acridotheres tristis*); Grey Partridge (*Perdix perdix*); Turkey (*Meleagris gallopavo*); *Clostridium perfringens*; cpb2; cpa.

**Abstract**

A non–enterotoxin (CPE)–producing *Clostridium perfringens* type A, associated with enteritis in a mynah (*Acridotheres tristis*), a grey partridge (*Perdix perdix*) and a turkey (*Meleagris gallopavo*) was characterized from cases with clinical symptoms from September 2010 until October 2012. Affected birds exhibited anorexia and diarrhea. Gross and histological findings were indicative of acute necrotic enteritis. *Clostridium perfringens* was isolated in bacterial cultures. Multiplex PCR for toxin profiling of the isolates revealed that the all three isolates were *Clostridium perfringens* type A, positive for cpb2 and cpa.

**Abbreviations**

*C. perfringens*: *Clostridium perfringens*
PCR: Polymerase Chain Reaction
SBA: Sheep Blood Agar
TSC: Tryptose Sulfite Cycloserine agar
TSN: Tryptose Sulfite Neomycin agar
CPA: *C. perfringens* Alpha toxin
cpb2: *Clostridium perfringens* beta2 toxin
netB: Necrotic Enteritis Toxin B
cpe: *Clostridium perfringens* Entrotoxin
etx: Epsilon Toxin
itx: Iota Toxin
TcdA: *Clostridium difficile* toxin A
TcdB: *Clostridium difficile* toxin B
TcsL: *Clostridium sordellii* lethal toxin
TcnA: *Clostridium novyi* alpha-toxi
Introduction

Necrotic enteritis is caused by Clostridium perfringens (CP), a gram positive, anaerobic bacterium which is commonly an inhabitant of the gastrointestinal tract of many mammalian and avian species [1-3]. This microorganism is recognized as an important pathogen in many species. It can cause gas gangrene and food poisoning in humans, necrotic enteritis in poultry species, enterotoxemia in calves and lambs, enteritis in cattle, dogs, pigs and horses [1-3]. The role of other recently investigated toxins such as Net B, TpeL and cpb2, remains to be completely defined [4]. Researchers have clarified the role of the β, ε and ι toxins in the pathogenesis of enteric diseases, but the exact role of the α-toxin is still controversial [2, 5, 6]. CP has been isolated from enteritis cases of different avian species like chickens, turkeys, ostriches and rarely psittacine birds [1, 7-13]. Nevertheless there is no report of isolation of CP from enteritis in some other species such as mynahs and partridges. Diets containing high concentration of proteins, polysaccharides and fat favor the intestinal environment for the Clostridium perfringens growth [14, 15]. Furthermore, stressful factors can also lead to occurrence of necrotic enteritis [13].

This study describes histopathologic, microbiologic and molecular toxintyping of three cases of NetB negative Clostridium perfringens infection associated with acute necrotic enteritis in a mynah (Acridotheres tristis), a grey partridge (Perdix perdix) and a turkey (Meleagris gallopavo).

Case description

Case 1: A one-month-old female mynah (Acridotheres tristis) presented with severe dysentery and anorexia. Symptomatic therapy was initiated, but clinical signs deteriorated and the animal was finally euthanized. At necropsy, small intestines were swollen, filled with gas, and thin walled. Diffused mucosal necrosis was also observed in large portions of small intestine which covered by a yellow-brown pseudomembrane.

Case 2: In a flock of 300 broiler turkeys (Meleagris gallopavo), 100 birds were died in a short period of time with clinical signs of diarrhea and lethargy. At necropsy, congestion and hemorrhage of the small intestine was observed in some of the recently dead birds. Tissue specimens were taken from intestines for histopathologic, microbiologic and molecular assessments.

Case 3: Followed by mortality in a flock of partridge (Perdix perdix), a 40-day-old male partridge with a history of diarrhea was referred for necropsy. At necropsy, there were hemorrhagic foci and congestion in the intestines.

Pathology

Necropsy and gross pathologic examination were carried out on all three cases. Small intestine samples, were fixed in 10% neutral buffered formalin, processed and stained with Hematoxylin and Eosin [16].

Bacterial isolation and characterization

Samples for bacterial isolation were obtained aseptically with sterile swabs from the gut, and were subjected to gram staining. Subsequently, intestinal sample was streaked onto blood agar plates containing 7% defibrinated sheep blood and incubated anaerobically at 37°C for 48 hr. Colonies which showed characteristic dual hemolytic zones were picked up and sub-cultured in Tryptose Sulfite Cycloserine agar (TSC) and Tryptose Sulfite Neomycin agar (TSN) for purification. The identity of the isolates was confirmed by their colonial and microscopic morphology, hemolytic pattern and Gram staining. All culture media and additives used in this study were provided from Merck (Germany). Reference strains of Clostridium perfringens: ATCC 13124 (cpa); CIP 106157 (cpa, cpe); CIP 60.61 (cpa, cpb, etx, cpb2) were used as positive controls. All bird treatments were conducted according to Animal Care Guidelines of the Research Committee, Faculty of Veterinary Medicine, Ferdowsi University of Mashhad.

Multiplex PCR

A single colony of the isolate was suspended in 100 μl distilled water, boiled for 10 min and then centrifuged at 10 000 g for 10 min. The supernatants were collected carefully and used as template DNA for PCR. Six pairs of primers were used to determine the presence of cpa, cpb, iA, etx, cpe and cpb2 genes using a multiplex PCR technique for all isolates [12, 23]. The primers and other materials used in PCR reaction were provided by Ampliqon [Odense, Denmark]. Amplification reactions were carried out in a 50 μl reaction volume containing 5 μl 10 x PCR buffer, 5mM dNTPs, 25 mM MgCl₂, 5 U of TaqDNA polymerase, 0.5 mM of each cpa oligo, 0.36 mM of each cpb oligo, 0.36 mM of
each cpb2 oligo, 0.52 mM of each ia oligo, 0.44 mM of each etx oligo, 0.34 mM of each cpe oligo and dH2O. Ten μl of template DNA was added to the mixture. Amplification was programmed in a thermocycler (Techne TC-3000, England) as follows: 95°C for 3 min followed by 35 cycles of 94°C for 1 min, 55°C for 1 min, 72°C for 1 min, and a final extension at 72°C for 10 min [13].

**TpeL single PCR**

PCR to detect TpeL was performed subsequently with 50 pM of primers [17]. PCR conditions included initial denaturation at 94°C for 5 min, 30 cycles of denaturation at 94°C for 1 min, annealing at 55°C for 1 min, and extension at 72°C for 1 min, and with a final extension step at 72°C for 7 min.

**Single PCR for netB:**

Previously developed primers were used to detect netB gene[18]. Reactions were carried out in a 25 μL reaction volume containing: 2 μL 10 x PCR buffer, 2.5 mM MgCl2, 0.2 mM dNTPs mixture, 2.5 units of Taq DNA polymerase, 0.1 μM of each primer, dH2O and 5 μL of DNA extraction solution. Amplification conditions were as follows: 94°C for 2 min followed by 35 cycles of 94°C for 30 sec, 55°C for 30 sec, 72°C for 60 sec and a final extension at 72°C for 12 min. Reference strains of Clostridium perfringens ATCC 13124 (cpa); CIP 106157 (cpa, cpe); CIP 60.61 (cpa, cpb, etx, cpb2) and two isolates JRMTK01 (netb+), and JRTK01 (tpeL+) were used as positive controls. The amplification products were detected by gel electrophoresis (Padideh NojenPars, Iran) in 1.5% agarose gel in 1x TAE buffer, stained with 0.5 μg/ml EtBr. Amplified bands were visualized and photographed under UV transilluminator.

**Results**

**Characterization of Bacterial Isolates**

Bacterial isolate exhibited the characteristic features of *Clostridium perfringens*. The colonial characters on blood agar showed dew drops, smooth, grayish, and convex colonies with a double zone of haemolysis. *Clostridium perfringens* formed black colonies in TSC and TSN agar. Microscopic characters revealed Gram positive non motile rods with box-car-shaped square cells. The Gram stain of tissue specimens from field cases of NE demonstrated that rod-shaped bacteria with typical *Clostridium perfringens* morphology formed large clumps primarily around the necrotic areas.

**Multiplex and single PCRs**

The *Clostridium perfringens* isolates from all tested birds were characterized as type A, positive for cpb2 (Figure 1) and negative for netB and tpeL.

**Histopathology**

Microscopic examination of case No.1 (mynah), revealed heavy infiltration of heterophils in the intestinal mucosal layer with severe hemorrhage and congestion. Severe necrosis and desquamation of enterocytes was also observed (Figure 2). Histopathologic lesions in case No. 2 (turkey) revealed necrosis of enterocytes along with destruction of intestinal villus. Hemorrhage and congestion were also observed (Figure 3). In case No. 3 (partridge), severe destruction of intestinal villi associated with hemorrhage and congestion were evident.

Results from pathologic, microbiologic and molecular examinations were representative of necrotic enteritis and the microorganism isolated was confirmed as *Clostridium perfringens* type
A. This bacteria has been widely studied in broiler and laying chickens and is known as a cause of mortality in flocks [13]. To the authors’ knowledge, there is not enough literature, about the exact prevalence of this microorganism in exotic birds and other minor avian species like partridges or even turkeys.

Discussion

_Clostridium perfringens_ is a ubiquitous bacterium found in the environment like soils and intestinal flora. So far, there are few reports of necrotic enteritis due to _Clostridium perfringens_ type A in turkey flocks [7]. Toxinotyping of _C. perfringens_ isolated from diseased and healthy turkeys, revealed that 100% of the isolates were positive for α-toxin, while just 6.6% of the isolates, which were just from necrotic enteritis cases, were positive for NetB. This phenomenon shows the importance of this gene in the pathogenesis of _C. perfringens_ in turkey, as in none of the isolates from healthy turkeys, this gene was identified. Nevertheless, it should be considered that 93.4% of the necrotic enteritis cases were negative for NetB, which indicates other pathogenesis factors of CP, may be involved in the above mentioned outbreak. In this study we described isolation and identification of a _Clostridium perfringens_ type A, as a cause of a huge mortality and loss in a broiler turkey flock.

To the authors’ knowledge there is no report concerning enteritis caused by _Clostridium perfringens_ type A in partridges. A similar report from red-legged partridges has been published in which _Clostridium perfringens_ type C was isolated. Occurrence of _Clostridium perfringens_ type A infection in game birds or exotic avian species is very rare, and most of the reports and studies are related to commercial flocks such as broiler chicks, ostriches and quails [7-11, 15, 19]. Our knowledge about pathogenesis of Clostridial infections in
NetB negative enteritis

birds are mostly based on studies in broiler chickens which might not be the same in other bird species [1, 7, 10, 13, 15].

In the present study, all isolates were positive for cpb2 and cpA and negative for cpb, iA, etx, cpe, NetB and tpeL genes. These results suggest that pathogenesis of the C. perfringens in the species discussed in this study may be different from those in commercial poultry chickens.

NetB and tpeL are two key virulent factors expressed by Clostridium perfringens to induce clinical diseases in chickens. While importance of NetB and tpeL in pathogenesis of clostridial necrotic enteritis in chickens has been shown [17, 18, 20-22], all isolates in this study were negative for NetB and tpeL. These findings suggest that clinical disease may appear even in the absence of tpeL and NetB genes. More studies are required to clarify the importance of different genotypes of Clostridium perfringens isolates, and the exact role of different toxins in the pathogenesis of necrotic enteritis in birds.

To our knowledge this is first report of occurrence and genotyping of necrotic enteritis caused by Clostridium perfringens type A in partridges (Perdix perdix) and Mynah (Acridotheres tristis).

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Author Contributions

Desinged the study and conducted the systematic literature review: J.R. and B.Sh. Performed pathological studies: A.R.M. and M.R.

Conflict of Interest

The authors declare that they have no competing interests.

References


12. Razmyar J, Kalidari GA, Tolooe A, Rad M, Movassagh AR. Genotyping of Clostridi-


Ovarian Fibrothecoma in a Holstein cow:
A Case report

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Keywords
bovine; Fibrothecoma; histopathology; Ovary; tumor

Abstract
A 5-year-old Holstein cow was referred to the Veterinary Medicine Hospital, Urmia University, Urmia, Iran, with abnormal estrous cycle. At rectal palpation, the unilateral ovary enlargement was detected. On transrectal ultrasonography view, the left ovary had uniformly hyperechogenic areas. The affected ovary was removed by ovarioectomy and sent for histopathological examination. Histopathological evaluation revealed fibroblastic cells producing collagen fibers and theca cells containing lipids. Based on histopathological features, diagnosis of fibrothecoma was confirmed. This case reports an extremely rare fibrothecoma in cow.

Abbreviations
MHz: Megahertz
HCl: Hydrogen chloride
GTCT: Granulosa-theca cell tumors
WHO: World Health Organization
mg: Milligram
Kg: kilogram

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Introduction

Ovarian tumors are uncommon in domestic animals. However, they have been reported more often in the mare, cow, bitch and chimpanzee [1]. Ovarian tumors are classified based on function, the histological features of tumor cells, similarity to normal cells, and embryological origins of the predominant cellular constituents [1]. Based on embryological origins, ovarian tumors can be related to three cellular subtypes: 1) the epithelium of the ovary consisting the surface modified mesothelium, (also called rete ovarii), for example in papillary and cystic adenomas and papillary adenocarcinomas. These cellular subtypes are less frequently involved in human and animal ovarian tumors; 2) the germ cells (dysgerminoma and teratoma) and; 3) the stroma of the ovary that includes the sex cords [1].

Ovarian fibrothecoma is a type of benign tumor of sex cord–stromal cell origin, but it is rarely malignant and includes both types of ovarian fibroma and ovarian thecoma cells [2]. Ovarian fibroma is a mesenchymal cell tumor which consists of fibroblasts and collagen. It seldom has thecal cells or estrogenic production. Fibromas has less cellularity and collagen content in comparison to fibrothecomas. Edema in organs is one of the main consequences of fibroids cancer which happens in more than 50% of the patients [3]. In comparison, ovarian thecoma which is a sex cord–stromal ovarian tumor, derives from normal cells constituting endocrine apparatus of the ovary. It contains lipid vacuoles with a small component of fibroblasts and has the capacity to produce steroid hormones. Due to histological overlapping between fibroma and thecoma tumors, the term fibrothecoma is used for describing a neoplasm that contains features of both thecomas and fibromas [4]. The present study describes the pathomorphology and ultrasonography findings of fibrothecoma in a Holstein cow presented to the Veterinary Medicine Hospital of Urmia University.

Case Presentation

A 5-year-old Holstein cow was referred to the Veterinary Medicine Hospital of Urmia University, Urmia, Iran being calved seventy-two days ago. The main complaints were abnormal estrous cycle and invisible estrus signs following two hormonal therapies. Perineal and vulvar conforma-
(vimentin and calretinin) were used to define the immune profile of the fibrothecoma [5].

**Results and Discussion**

Vaginoscopy indicated that vulvar and perineal regions were normal, however, the vulvar region was edematous. Uterus and right ovary were normal in size and consistency and no remarkable

![Figure 3](image3.png)

**Figure 3**
Macroscopic image of the cut of the ovarian fibrothecoma, surrounded by thick and firm consistency wall.

![Figure 4](image4.png)

**Figure 4**
Microscopic examination of ovarian fibrothecoma shows hypercellularity, with two cellular patterns; as seen with higher magnification in right corner (H&E; 400x), fibroma that contained spindle-shaped cells with longed nuclei in a fibrocollagenous stroma (white arrows), and the thecoma that contained compressed oval-round or fusiform cells with vacuolated cytoplasm (dark arrows); (H&E; 100x).

![Figure 5](image5.png)

**Figure 5**
Fibrothecoma tumor cells are positive for vimentin (A) and Calretinin (B) (Immunohistochemistry, 400x).
structure (follicle or corpus luteum) was recognized during transrectal palpation. In contrast, the left ovary was massive and smooth. On transrectal ultrasonographic imaging, uterus (without edema) was normal, but there were small abscesses in the middle part of the cervix. The left ovary had uniformly hyperechogenic areas on the ultrasonography (Figure 1). Based on the rectal palpation and ultrasonographic findings, the left ovary tumor was suspected.

It was removed with standing laparoscopic ovariectomy method. Left-flank approach in caudal area of paralumbar fossa was scraped, shaved and made ready for operation. L-block anesthesia was used and inverted with 10 ml of lidocaine HCl 2% (0.22 mg/kg; 5.5 ml/500 kg) (Pasteur Institute, Tehran, Iran) [6]. After ovary was externalized on peritoneum cavity and exposed to proper space, the mesovarium was ligated with 2 polyglycolic acid (Supa, Tehran, Iran), by using a sliding half-hitch knotting technique. The ovary was removed with mayo scissors over the ligatedregion and the abdominal wall was closed with a 3-plan suture in a simple continuous pattern with 2 polyglycolic acid.

In macroscopic study, the tumor weighted 254 grams and solid mass diameter was approximately 4.67×4.87×3.03 cm. The outer surface of the ovary was smooth (Figure 2). After the tumor was opened, we noticed that it was surrounded by a thick, firm, and consistent wall and the interior section was composed of a solid mass along with a necrosed and a hemorrhaged part (Figure 3). Histologically, a benign neoplasm was observed which consisted of two cellular patterns. The stroma revealed a dense fibromatous area that was composed of fibroblastic cells in an interwoven pattern (Figure 4). Results of immunohistochemical staining delineates that the spindle-shaped neoplastic cells were positive to vimentin (Figure 5A), while tumor cells were strongly positive for calretinin (Figure 5B).

**Discussion**

Fibromas and Fibrothecomes are the sex cord-stromal origin tumors [1]. In addition, according to WHO (World Health Organization) classification of ovarian tumors, they are a sub-group of granulosa-theca cell tumors (GTCT) which belong to the thecoma-fibroma group [7].

In this study, fibrothecoma was detected after ultrasonography, histopathological examination, and rectal palpation. Conte and colleagues examined 11 cases of fibrothecoma and suggested some diagnostic sonographic patterns [4]. The presence of homogeneous echogenic patterns that marks the posterior acoustic shadowing along with the lack of calcification, is highly suggestive that a fibrous ovarian fibrothecoma exists.

To the best of our knowledge, this is the first fibrothecoma report in cow, but there were several reports about other animals. Ovarian tumors in mares are almost unilateral and are present at an incidence of 5-6% in comparison to other ovarian tumors and the fibrothecomas is extremely rare in this sub-group [8]. Changes in behavior will happen in mares in case of tumor’s activity and hormonal production, and it should be noted that high estrogen secretion by thecomal section of tumor leads to behavioral changes. Abnormal clinical signs such as defects in ovulation or implantation may appear which lead to infertility, prolonged anestrus, aggressiveness, masculine behavior, or nymphomania [9]. Azizi et al. reported an ovarian fibrothecoma in a 10-year-old Arabian mare that had a similar gross lesion and microscopic feature as shown in the present study [10]. Jorritsma and colleagues has reported granulosa-theca tumor in a 2-years-old cow [11]. The case didn’t show pregnancy after artificial insemination and entered into estrus again, but after the blood and histopathological examination thecoma tumors was diagnosed. This case and another similar case reported by Tontis and colleagues had nonspecific and definitive diagnostic clinical signs similar to our report and the tumor classified as a Thecomas after histopathological examination [12]. Different types of solid benign ovarian cysts and tumors, such as Brenner tumor, thecoma, fibroma, and fibrothecoma, may manifest similar echogenicity on ultrasonography [13]. Thus, it is suggested that other more specific tests such as, histopathological evaluation and immunohistochemical examination [14] should be performed in order to obtain a more accurate diagnosis [15]. Immunohistochemically, the tumor cells in this case were strongly positive for calretinin and vimentin.

In conclusion, the cow in this report, with an enlarged ovary includes a tumor that has metastasized to the ovary. To the best of our knowledge, it is the first ovarian fibrothecoma reported in Holstein cow which was confirmed by histopatholog-
Ovarian Fibrothecoma in a Holstein cow

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Author Contributions

Conceived and designed the experiments, performed the experiments, and wrote the paper: A.S., R.B., A.N. and B.P. Analyzed the data: A.S.

Conflict of Interest

The authors declare that they have no conflict of interest.

References


چکیده
تب شیر و هپیوکلسیمی تحت بالینی اختلال پیسار مهم مواد معدنی در دوره انتقال گاو شیری است. مطالعات متعددی نشان داده است که گاه‌هاي مبتلا به هپیوکلسیمی نسبت به اختلالات و بیماری‌های زایده مستعد هستند. در اولین دوره شیردهی خروج قابل توجه کلسیم از بدن اثر شبه‌افزایش چشمگیر اختصاصی بنده کلسیم می‌گردد. این نسبت به اواخر دوره ابستن و نیازهای معقول بنده کلسیم خیلی پیشتر است. نیاز غدد پستانی به کلسیم از توان دام برای چاپ‌گیری کلسیم از دست رفته پلاسما فراتر می‌رود. کلسیم خون در حوالی و حوش راهی یافتده و در 12 تا 24 ساعت اول پس از زایمان به کمترین مقدار خود میرسد. برای حفظ هموستان کلسیم، مکانیسم‌های جیرانی در اولین راهی فعال می‌گردد. این مکانیسم‌ها شامل فعالیت هموسیت های 1 و 2 دی‌هیدروکلسی و نتایج D3، هموسیت‌های بارتروری و هموسیت‌های گلوکائی فعالیت دارند. در المانده می‌توان این مکانیسم‌ها از مصرف بدن بهره‌وری کند و به راحتی به هپیوکلسیمی در بالینی مبتلا به گلوکائی و انتقال آب و الکل را بهبود بخشیده و نیز نیازهای بالینی کلسیم را تأمین نماید.

کلید واژه‌ها: تب شیر، هپیوکلسیمی، تحت بالینی، گاو شیری، پایش

Abstracts (In Persian)

Persian Abstracts

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هپیوکلسیمی تحت بالینی در گاو شیری: پاتوفیزیولوژی، عواقب و پایش

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چکیده

تب شیر و هپیوکلسیمی تحت بالینی اختلال پیسار مهم مواد معدنی در دوره انتقال گاو شیری است. مطالعات متعددی نشان داده است که گاه‌هاي مبتلا به هپیوکلسیمی نسبت به اختلالات و بیماری‌های زایده مستعد هستند. در اولین دوره شیردهی خروج قابل توجه کلسیم از بدن اثر شبه‌افزایش چشمگیر اختصاصی بنده کلسیم می‌گردد. این نسبت به اواخر دوره ابستن و نیازهای معقول بنده کلسیم خیلی پیشتر است. نیاز غدد پستانی به کلسیم از توان دام برای چاپ‌گیری کلسیم از دست رفته پلاسما فراتر می‌رود. کلسیم خون در حوالی و حوش راهی یافتده و در 12 تا 24 ساعت اول پس از زایمان به کمترین مقدار خود میرسد. برای حفظ هموستان کلسیم، مکانیسم‌های جیرانی در اولین راهی فعال می‌گردد. این مکانیسم‌ها شامل فعالیت هموسیت های 1 و 2 دی‌هیدروکلسی و نتایج D3، هموسیت‌های بارتروری و هموسیت‌های گلوکائی فعالیت دارند. در المانده می‌توان این مکانیسم‌ها از مصرف بدن بهره‌وری کند و به راحتی به هپیوکلسیمی در بالینی مبتلا به گلوکائی و انتقال آب و الکل را بهبود بخشیده و نیز نیازهای بالینی کلسیم را تأمین نماید.

کلید واژه‌ها: تب شیر، هپیوکلسیمی، تحت بالینی، گاو شیری، پایش
تغییرات هیستوپاتولوژیک در آلودگی تجیری با پیادروس فاسیس در رت

چکیده
جنس پیادروس جدید 621 گونه را شامل می‌شود که با همه گیری‌های درمانیت به‌دست آمده است. هدف از این مطالعه مشخص نمودن تغییرات ماکروکمپویی و میکروکمپویی تولید شده بوسیله پیادروس فاسیسی بود. سوسک‌های بالغ پیادروس فاسیسی از خانه‌های آلوده جمع‌آوری و به آزمایشگاه فرستاده شد. سوسک‌ها در ناحیه تراشیده پلاک شده‌اند رت داخل یک حلقه پلاستیکی گذوری است. پس از اکثریت گود شده بود، قرارداده شدند. در گروه دوم حشرات به شیوه ناحیه اوریک گوش خارجی رنگ را مالیده شد. تغییرات ماکروکمپویی بعد از 12 ساعت مورد بررسی قرار گرفت که شیب پایه‌ای ارتشتام بود. پس از 24 ساعت نواحی مذکور بزرگتر و متركب‌تر شد. آزمایشات میکروکمپویی نشان دهنده ادم و انتخاب خفیف سلول‌های التهابی بود. پس از 12 ساعت ادم و تغییرات هیدروپیک در سلول‌های بازال و اسکوادر سپس از 24 ساعت و تخریب ابهرم‌های تجمع شدید مایع همراه با تشکیل ورزگر پس از 72 ساعت بود. در این مطالعه نتایج ماکروکمپویی و میکروکمپویی عمداً در رت‌های مشاهده گردید که با موارد حاصل از لاش‌گردی میکروکمپویی و زنده نشان دهنده ادم ناحیه در همراه با ارتشتام سلول‌های التهابی لفositی و انتزوفیلی بود.

واژگان کلیدی: سوسک، پیادروس فاسیس، درمانیت، هیستوپاتولوژیک، رت
بررسی آلودگی به ویروس لوسمی گربه‌سانان (FeLV) در گربه‌های شهرستان اهواز، ایران:
شیوع سرمی و فاکتورهای خطر

فازنده زرمینی شهرکبک، عمیشه پوراندیش، پهلوانی، سیدمحمدی، مشهدی احمدی

چکیده
هدف از انجام مطالعه حاصل، تبیین میزان شیوع سرمی ویروس لوسمی گربه‌سازان در گربه‌های شهرستان اهواز، واقع در جنوب غرب ایران بوده، همچنین فاکتورهای خطر توزیع سن، جنس، تعداد نوزادان گربه و بیماری‌های بیماری‌های لازم‌بودن. نمونه‌برداری از 60 گربه خانگی، 124 گربه دامداری و 66 گربه فردی از گروه‌های مختلف و با استفاده از کیت ال آر آی، عبارت از آنتی‌بادی ضد ویروس لوسمی در گربه‌سانی سنجیده شد. شیوع سرمی در جمعیت گربه‌های 29/6/89 درصد (با فاصله اطمینان 95 درصد، 74/1-68/8) درصد) از گروه جوان و 13/38 درصد (با فاصله اطمینان 95 درصد، 74/1-68/8) درصد) از گروه بزرگسال مواجه بودند. آزمون تکراری کاپی نشان داد که ارتباط معنی‌داری بین سن‌های مرجعی و آلودگی وجود دارد (P<0.01). الگویی در رده سنی 2 سال و پایین‌تر به مراجعی داری کمتر از رده سنی 3-4 سال (P<0.05) و باقی 4 سال (P<0.10) بود. میزان شیوع سرمی در گربه‌های دانشکده پزشکی تهران، از پرستاری تا از پرستاری بود (P<0.05). سپس الگویی در گربه‌های دانشکده پزشکی، با بین وجود تفاوت از نظر آماری معنی‌داری دار نبود (P>0.05). در قسمت نتیجه‌گیری، شیوع معنی‌داری در جمعیت گربه‌های منطقه اهواز، بسیار بالا بود و نتایج معنی‌داری بین یافته‌های دانشگاهی و نتایج سرولوژیکی، وجود داشت.

واژگان کلیدی: سرولوژی، شیوع، ویروس لوسمی گربه‌سانان (FeLV)، گربه، اهواز

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دریابی مایکوپلاسمای بویس در نمونه گرفته‌شده از مخزن شیر گاو‌داری‌های مشهد بای روش nested PCR

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چکیده

مایکوپلاسمای بویس یک باکتری مهم و بسیار واقع و پیشان است که تهیه‌دهنده گاه‌های ایجاد می‌کند. این نوع از ورم پستان به این بیوتیک‌های دردسرس پاسخ نمی‌دهد و در واقع درمان مولی برای این نوع از عفونت وجود ندارد. بنابراین بهترین راه جلوگیری و کنترل این نوع ورم پستان مناسب به حذف گاه‌های اوده از گل است. هدف این مطالعه دریابی مایکوپلاسمای بویس در نمونه‌های شیر از طریق شده‌اند از مخزن شیر گاو‌داری‌های اطراف مشهد بای روش nested PCR می‌باشد. مجموعه یکصد و وان نمونه شیر ناری از ۳۵ گال شیر شیری با فاصله چهار هفته از یکدیگر اخذ گردید. PCR modified hayflick مایکوپلاسمای بویس از هیچ گذار از نمونه‌ها با روش PCR مستقیم بر روز شیر و یا پس از غنی‌سازی در حیطه مورد نظر دو گونه از مایکوپلاسمای بویس از فردوسی مشهد، با استفاده از PCR لنوکلوترونیک دو روز به منظور تعیین نوکلوترونیک بر روی محصولات مایکوپلاسمای بویس (Mycoplasma yeatsii) و مایکوپلاسمای کانادئ (Mycoplasma canadense) و انجام PCR مغناطیسی بر روی نمونه‌های مشتد انجام گرفت و حضور دو گونه مایکوپلاسمای بویس (Mycoplasma yeatsii) و مایکوپلاسمای کانادئ (Mycoplasma canadense) را تایید نمود. واژگان کلیدی: مایکوپلاسمای بویس، شیرخانه، مشهد، ایران.
چکیده

در این تحقیق خصوصیات باسخهای ایمنی واکسن‌های کابری باکس ویروس بس زا اضطراری کاوه بر عیله ویروس بیماری لمی اسکین

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خطای تصادفی: P<0.05

یک هزار و بیست و یک نهایی از واکسن‌های ایمنی سلولی نشان داد که تکثیر لنفوسیت‌ها و تولید سابتوکاین‌های Y و IFN-γ و IL-4 در هر دو گروه واکسین‌گذاری و در هر دو گروه واکسین‌گذاری بر اساس مقادیر خود رسمی و در گروه‌های دو واحد از این نتیجه بایستگی از نظر این نتایج بیشتر از گروه‌های واکسین‌گذاری شده با واکسن آبژریزی سپار بینشر از گروه‌های واکسین‌گذاری شده با واکسن آبژریزی سپار بینشر و در هر دو گروه واکسین‌گذاری بر اساس مقادیر خود رسمی و در گروه‌های دو واحد از این نتیجه بایستگی از نظر این نتایج برای بینشر دارای احتمال: P<0.05

این یافته‌ها نشان داد که واکسن GPV به دلیل اقای مقاومت بالاتری از تنری سرم انتی باید تحریک لنفوسینی و تولید سابتوکاین‌های Y و IFN-γ و IL-4 نسبت به واکسن SPV، دارای خصائص ایمنی‌ای پیش‌ریز می‌باشد. به طوری که می‌تواند به عنوان واکسن مناسب برای کنترل و پیشگیری از بیماری لمی اسکین در نظر گرفته شود.

واژگان کلیدی: آبژریزی، آناتومی، واکسن، IFN-γ، IL-4
چکیده

آدنوپروس‌های پرندگان به عنوان گروه متنوعی از بیانگز را شناخته می‌شوند. این ویروس‌ها مهم‌الثبوتی
و سبک از علائم بالینی را ایجاد کرده‌اند که این مال‌کام‌بدون علایمت باشد. هدف از انجام این مطالعه، شناسایی
مولکول‌های آدنوپروس‌های پرندگان در کل‌های مایکان گوشتخانه‌های مختلف درنوسندهای (IBH) و
سندروم‌های نفیسی در جنوب غربی ایران بوده‌اند. این منظور، نمونه‌های کبد و ریه که در زمان اخیر
پیدا شده‌اند از گروه کشتارگاهی در جنوب غربی ایران، تهیه شدند.

در صورت انتخاب شد و سپس تحت آزمایش قرار گرفتند، مورد بررسی قرار گرفتند. نتایج بدست آمده از آزمایش
NCBI (مورد بررسی قرار گرفت، نتایج دست‌آمده از آزمایش NCBI که ترکب شده از یک گل‌های 25 روزه بود. در اساس اطلاعات به دست آمده از توالی نوکلوئیدی این توالی متعلق
به زنیت‌های D آدنوپروس‌های بود. در مقایسه این توالی با جدایی‌های نسبی شده در مینه‌زایی، این ویروس با سروریپ
11 آدنوپروس‌های جدا شده در کشورهای ایران، چین، کانادا و استرالیا تا 99 درصد تطبیق داشت. تحقیق حاضر
اخلاصه مطالعه در زمینه دیابی مولکولی و بررسی نوکلوئیدی توالی فلزه‌ی زن هگزبن آدنوپروس پرندگان در
کل‌های مایکان گوشتخانه جنوب غربی ایران می‌باشد.

واژگان کلیدی: آدنوپروس پرندگان، مایکان گوشتخانه، IBH، سروریپ 11 جنوب غربی ایران

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چکیده

آزمایش ۶۳ روزه‌ای تحت شرایط کنترل شده جهت مقایسه اثرات فروکتوالیگوساکارید در چهار سطح (۵، ۱۰، ۲۰ و ۳۰ گرم بر کیلوگرم) بر عملکرد رشد، شاخص‌های کارایی تغذیه، بارامترهای هماثوایمنولوژیکی، مقاومت به استرس، انزیم‌های گوارشی و فلور میکروبی کلی انجام شد. ماهیان Schizothorax zarudnyi تحت شرایط کنترل شده به میزان متوسط وزنه ۵۲/۱ ± ۵۲/۶۸ گرم به طور میانگین دارای وزن نهایی برابر با ۶۸۳/۲ ± ۵۹/۳ گرم بودند. استفاده از فروکتوالیگوساکارید (۵-۲۰ گرم بر کیلوگرم) اثرات معنی‌داری (p<0/05) بر وزن اصلی و وزن دسته در مقایسه با تیمار کنترلی داشت. ماهیان تغذیه شده با جیره حاوی ۲۰ گرم بر کیلوگرم فروکتوالیگوساکارید دارای نسبت بقاء بیشتری نسبت به تیمارهای دیگر بودند. علاوه بر این، استفاده از فروکتوالیگوساکارید به طور معنی‌داری اثرات معنی‌داری (p<0/05) بر کمیت و کارایی غذا و انرژی سلولی در ماهیان تغذیه شده را افزایش داد. میزان CMP، MCH، MCV و همچنین مقاومت به استرس و مقاومت به تغییرات محیطی در ماهیان تغذیه شده از جیره حاوی فروکتوالیگوساکارید (۳۰ گرم بر کیلو گرم) به طور معنی‌داری افزایش یافت. نتایج حاضر نشان داد که استفاده از فروکتوالیگوساکارید به طور معنی‌داری اثرات منفی بر فلور میکروبی روده و میزان چکیده را افزایش داد.

واژگان کلیدی: Schizothorax zarudnyi، فروکتوالیگوساکارید، رشد، بارامترهای هماثوایمنولوژیکی، میکروب‌ها روده
Abstracts (In Persian)

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ارزیابی حسی رنگ گوشت کامپیوتر با استفاده از سیستم بینایی کامپیوتر

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چکیده

ارزیابی حسی رنگ گوشت با استفاده از سیستم بینایی کامپیوتر به عنوان روشهای مطمئن برای یافتن مشکلات پیشرو در هنگام ارزیابی گوشت به صورت مستقیم خاکه وجود. در این مطالعه، در سه مدل گونه، ۳ نمونه علیه لابی‌کردنی دورسی از اشعه‌های مختلف در کشاورزارگاه نهایی به فضاهای با ضخامت ۵ میلی‌متر برداشم داده شدند. با مقایسه با استفاده از سیستم بینایی کامپیوتر در شرایط تصویربرداری استاندارد از بررسی عکس‌برداری انجام شد. همچنین همزمان، نرگ هنری‌ستان گوشت به وسیله رنگ زنی‌سنج هانترل (Hunterlab) تعبیه گردید. اولین عکس از نمونه، با مقایسه با اسیدهای گوشت با استفاده از عکس‌های گازاه دیده در طی ۱۳ روز از اضافه کننده به آنها با ترتیب اولویت انتخاب شد. جمع امتیازات با استفاده از آزمون فردوزه مورد ارزیابی قرار گرفت. به طور کلی، افراد ارزیاب مقدار روش‌نامه‌ای با (L*، C* و زردی (b*) نسبت با را در نمونه‌های گوشت را از بین روز پس از کشته ترجیح دادند.

واژگان کلیدی: سیستم بینایی کامپیوتر، رنگ گوشت، ارزیابی حسی، گوشت کامپیوتر
بررسی آنتی‌بودن نکروتیک ناشی از کلسستریدیوم پرفربینجنس فاقد زن تن بی در میان کیک خاکستری و پولکمون

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گروه پاتوبیولوژی، دانشگاه کوشک، گوئلف، انتاریو، کانادا

دریافت مقاله: 1396/10/30

پذیرش نهایی: 1396/12/29

چکیده

در این مطالعه کلسستریدیوم پرفربینجنس تیپ A فاقد زن انتروتوکسین از موارد آنتی‌بودن نکروتیک در یک میان کیک خاکستری و یک پولکمون با علائم درمان‌ناپذیر مشخصه بیماری آنتی‌بودن از شهریور 1397 تا آبان 1397 مورد بررسی و تعبیه شد. هوت قرار گرفت. برندگان مبتلا دارای علائمی چون، پاچای و اسهال بودند. شیوعات ماکرو‌سکپی و ماکرو‌سکپی نشان دهنده آنتی‌بودن نکروتیک حاد بود. کلسستریدیوم پرفربینجنس بصورت خاصی از پایگاه‌های درمانی شد و بر اساس نتیج پچت PCR B به گردید. نتایج PCR B داشتند.

واژگان کلیدی: میان کیک خاکستری، پولکمون، کلسستریدیوم پرفربینجنس، cpb2 و cpb
فواید کمک‌گذاری تخم‌دان در گاو هله‌شتاین: گزارش موردنی

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دریافت مقاله: 05/11/1395 پذیرش نهایی: 06/04/1396

چکیده
یک گاو 5 ساله هله‌شتاین با سیگنال علیه غیرطبیعی به درمانگاه دانشگاه ارومیه ارجاع شد. در معاگه و لکت تخم‌دان بصرت یک‌طرفه مورد توجه قرار گرفت. در بررسی اولترا صوئوگرافی رکتا، تخم‌دان سمت چپ در دارای اکوزن بود. تخم‌دان با علیه جراحی برداشته و برای ارزیابی پاتولوژی فرستاده شد. ارزیابی پاتولوژی نشان داد که ساختر فیبرهای کلاژن سلول فیبرولاستیک و سلول‌های تکا حاوی چربی بود. بر اساس یافته‌های بافت‌شناسی فیبروتومی تایید شد. در این کیفیکت فیبروتومی بسیار نادر در گاو گزارش شده است.

واژگان کلیدی: فیبروتومی، تومور، تخم‌دان، گاو، هله‌شتاین.
List of reviewers (2017, Volume 9, No. 1 and 2)

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Guide for Authors

Scope

Iranian journal of Veterinary Science and Technology (IJVST) is a peer-reviewed and multi-disciplinary journal that supports important research advances in veterinary medicine and subject areas relevant to veterinary medicine including anatomy, physiology, pharmacology, bacteriology, biochemistry, biotechnology, food hygiene, public health, immunology, molecular biology, parasitology, pathology, virology, etc. Contributions related to clinical sciences including large and small animal medicine, poultry disease, diseases of equine species and aquaculture are welcomed. Articles can comprise research in basic sciences, as well as applied veterinary findings and experimental studies with impacts on diagnosis, treatment and prevention of animal diseases.

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5. Ethics: Authors must state that the protocol for the research project has been approved by the Ethics Committee of the institution within which the work was undertaken. Authors are responsible for animal welfare and all statements made in their work.

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Review Articles should provide an update on recent advances in a particular field. Authors wishing to submit review articles should contact the Editor with an outline of the proposed paper prior to submission.

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Guide for Authors

INTRODUCTION

Introduction should be as concise as possible, and clearly explain the main objective and hypothesis of the investigation.

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Results indicate the results of an original research in a clear and logical sequence. Do not repeat data that are already covered in tables and illustrations. In manuscripts describing more than one animal, all animals should be assigned a case number.

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Discussion should include the answer to the question proposed in the introduction and emphasize the new and important aspects of the study and the conclusions that follow from them. It could include the implication, application, or speculation of the findings and their limitations, relate the observations to other relevant studies, and links the conclusions with the goals of the study. Recommendations, when appropriate, may be included.

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ACKNOWLEDGEMENTS

Personal acknowledgement, sources of financial support, contributions and helps of other researchers and everything that does not justify authorship should be mentioned in this section, if required.
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Conceived and designed the experiments: HD, SS. Performed the experiments: SS. Analyzed the data: HD, SS, MMM, ARB. Research space and equipment: HD, MMM, ARB. Contributed reagents/materials/analysis tools: HD. Wrote the paper: SS, HD.

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An unhealthy diet, obesity and physical inactivity play a role in the onset of type 2 diabetes, but it has been shown that increased physical activity substantially reduces the risk [1], and participation in regular physical activity is one of the major recommendations of the evidence-based guidelines for the primary prevention of diseases [2]. According to the 2004-05 National Health Survey, more than half a million Australians (3.5% of the population) have diabetes mellitus which had been medically diagnosed and most of these people have the Type 2 condition [3]. Gestational diabetes is also on the increase, rising steadily between 2000-01 and 2005-06 [4]. Approximately two thirds of those with diabetes have been prescribed medication [3], but it is of concern that a recent review of the literature found that many people do not take their medication as prescribed [5]. Many patients also self-monitor the disease by measuring their blood glucose levels with a glucose meter but Song and Lipman [6] have concerns about how well this is managed.

References


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Book [print], Single Author


Book [print] 2-6 authors

1. Eckerman AK, Dowd T, Chong E, Nixon L, Gray R, Johnson S. Binangoonji: bridging cultures in Aboriginal health. 3rd ed. Chatswood,
NSW: Elsevier Australia; 2010.

**Book [print] More than 6 authors**

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**Thesis**
1. Ho SH. Preventative risk modelling and mapping of Murray Valley encephalitis virus and dengue virus in Western Australia [master’s thesis]. [Perth (AU)]: University of Western Australia; 2015.

**Thesis Accessed from Library-subscribed database**

**Thesis Accessed on the web**
1. Seale AC. The clinical and molecular epidemiology of streptococcus agalactiae in Kenya:

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3. References/Bibliography Vancouver Style [Internet]. Perth: University of Western Australia; [cited 2017 Mar 6]. Available from: http://guides.library.uwa.edu.au

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